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E. E. GREEN, Way's End, Camberley (late Government Entomologist, Ceylon)

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VOLUME VII FEBRUARY, 1921 No 4

# NOTES ON A CESTODE OCCURRING IN THE HAEMOCOELE OF HOUSE-FLIES IN MESOPOTAMIA.

BY J. H. WOODGER, B.Sc.

*Assistant in Zoology, University College, London, sometime Protozoologist in the Central Laboratory, Amara.*

(With 3 Text-figures.)

## 1. INTRODUCTION.

THE parasite briefly described in the following notes was first observed by me in the course of a series of dissections of house-flies undertaken in conjunction with Capt. P. A. Buxton, R.A.M.C., in Amara, Mesopotamia, with a view to discovering to what extent the house-fly was responsible for the carriage of dysentery in that region. The results of this dysentery work have been published by Capt. Buxton elsewhere.

## 2. MODE OF OCCURRENCE IN THE FLY.

It was noticed one day, on opening the abdomen of a fly, that a number, about fifteen, of milky, globular bodies, apparently unconnected with the organs of the insect, poured out into the saline in which the dissection was being carried out. From the small amount of material obtained it is not possible to say more than that the parasite occurred free in the abdominal haemocoele.

## 3. LOCALITIES IN WHICH INFECTED FLIES WERE FOUND.

Flies were taken for this work from near British and Indian hospital latrines; from Arab compounds in and near Amara; from a village known as Deffas, about $2\frac{1}{2}$ miles above Amara town, on the right bank of the Tigris; and from what may be described as the "dirty end" of the Amara bazaar.

As this parasite was not found in any of the flies from British or Indian latrines these localities need not be considered further. They were kept clean under the supervision of the sanitary sections of the R.A.M.C.

The remaining localities mentioned were, for the most part, in a very filthy state. The Arab compounds and villages were usually packed with live-stock—horses, cows, dogs, fowls, sparrows, rats, and lizards—and their excreta—all providing ideal conditions for the breeding of flies and the transmission of parasites.

The "dirty end" of the bazaar was open to the sky and crowded with the stalls of Arab vendors of meat, vegetables, and sweetmeats; it was always swarming with flies, but live-stock was, of course, very much less in immediate evidence than in compounds and villages.

### 4. Season and Incidence.

It will be seen from the table below that the dissections of flies from the regions mentioned were continued from March 9, 1918, until September 26, 1918, with breaks during May and August. It will be noticed at once that flies infected with the cestode were only found during the month of April. After the 30th of that month frequent examination of flies from the same localities failed to yield a single parasite.

*Table of Examinations.*

| Date 1918 | Number of flies examined | | Locality | Result |
|---|---|---|---|---|
| | Male | Female | | |
| March 9 | — | 7 | Bazaar | Nil |
| „ 26 | 9 | 6 | „ | Nil |
| „ 28 | — | 1 | Deffas | Nil |
| „ 30 | 15 | 4 | Compound near Amara | Nil |
| April 2 | 23 | 19 | Bazaar | 2 flies infected. Sex not recorded |
| „ 3 | 11 | 8 | Compound near Amara | Nil |
| „ 17 | — | 7 | Deffas | Nil |
| „ 18 | — | 21 | „ | Nil |
| „ 23 | — | 17 | Compound in Amara | 1 female fly infected |
| „ 24 | — | 15 | „ „ | 1 female fly infected |
| „ 25 | — | 11 | „ „ | Nil |
| „ 29 | — | 14 | „ „ | Nil |
| „ 30 | — | 11 | „ „ | 1 female fly infected |
| June 8 | — | 3 | „ „ | Nil |
| „ 15 | — | 10 | „ „ | Nil |
| „ 20 | — | 20 | „ „ | Nil |
| „ 21 | 15 | 15 | „ „ | Nil |
| „ 24 | 10 | 14 | „ „ | Nil |
| July 6 | — | 10 | „ „ | Nil |
| Sept. 18 | — | 4 | „ „ | Nil |
| „ 21 | — | 13 | „ „ | Nil |
| „ 26 | — | 25 | „ „ | Nil |

The total number of infected flies found was only five out of the 338 dissected from the localities mentioned (*i.e.* all except British and Indian latrines),—a percentage of nearly 1·5 per cent.

### 5. DESCRIPTION OF THE PARASITE.

The following description and accompanying figures are taken from live specimens examined fresh in saline. Three developmental stages are

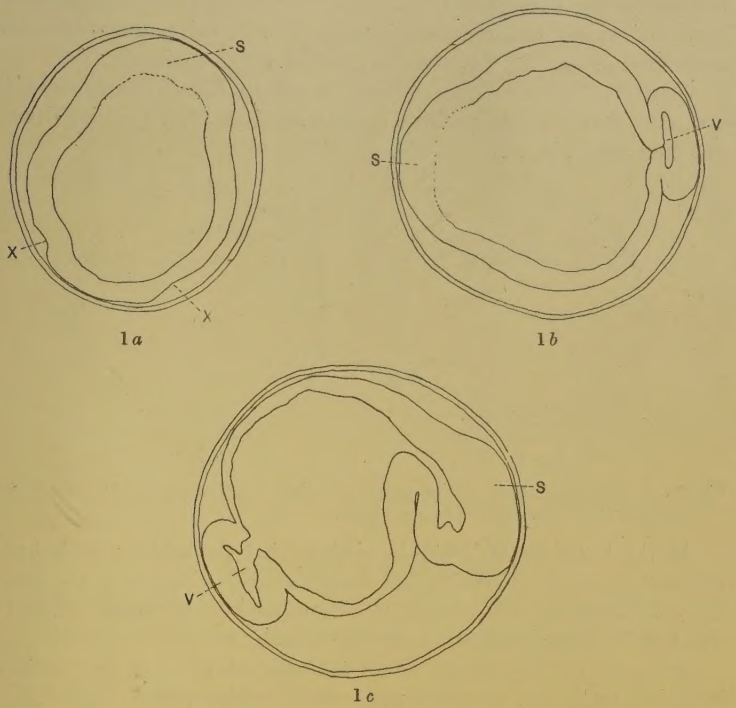


Fig. 1 *a*. The parasite in Stage I: *S*, scolex, *X*, points at which constrictions are appearing.
*b*. Parasite in Stage II: *S*, scolex, *V*, vesicle.
*c*. The same parasite, drawn one hour later.

represented, the specimens of each being from different flies, since all the parasites from the same fly were found to be in approximately the same stage. All figures magnified approximately 145 times.

*Stage I.* Fig. 1 *a* represents the earliest stage seen. The cyst-wall enclosing the embryo appears to consist of a single transparent, gela-

tinous envelope. The embryo itself consists of a thick-walled vesicle in which two distinct poles are recognisable. At one pole, *S*, destined to form the scolex, the wall is very thick, and its inner margin is difficult to make out, as is indicated by the dotted line. The other pole is distinguished, not by any striking difference in its wall, but by the signs of the beginning of a constriction at the points marked *X*.

During life slow waves of contraction are continually passing over the animal and consequently its shape may change very considerably. Fig. 2 is intended to illustrate this point, Fig. 2 *b* being drawn from the same specimen as Fig. 2 *a*, after an interval of three minutes.

*Stage II.* This is figured in Figs. 1 *b* and 1 *c*. These two figures also are drawn from a single specimen at different times, Fig. 1 *c* being drawn one hour after Fig. 1 *b*.

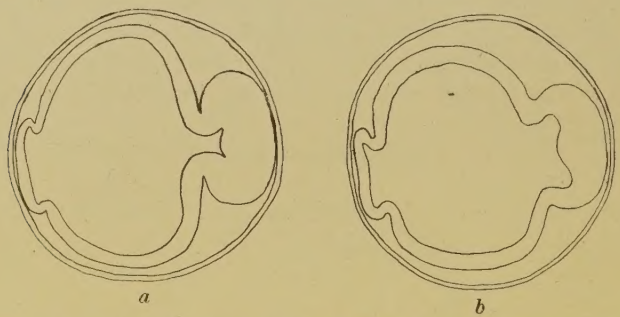


Fig. 2 *a*, *b*. Two drawings of another parasite, to show waves of contraction, taken with an interval of three minutes between them.

In the latter figure it will be noticed that the scolex-pole is little different from that of Fig. 1 *a*, but the constriction observed in the latter has now deepened so far as almost to separate off a flattened vesicle *V*. The cyst itself has increased somewhat in size.

After one hour we see, in Fig. 1 *c*, that the pole carrying the vesicle *V* is little altered, but at the opposite pole a thick-walled, club-shaped "head" is now marked out and folded over to one side. Remembering what has already been said concerning the rhythmical contractions of the animal it will be realised that it is difficult to say how far this difference between the scolex-pole of Fig. 1 *b* and of Fig. 1 *c* was due to these contractions or to an actual advance in development.

*Stage III.* This is represented by two parasites from one fly drawn in Fig. 3.

The entire cyst has now increased considerably in size. The vesicle

is entirely separated from the parasite. The head, attached by a narrow neck to the somewhat shrunken bladder, bears four suckers and a club-shaped rostellum, armed with a single row of small hooklets.

It will be noted that there are certain differences between the two specimens figured in Fig. 3. The hooklets are smaller and more numerous in one than in the other; and the shape of the rostellum is also different in the two specimens, but this is probably merely the effect of different conditions of contraction. The separated vesicle *V* also is smaller in one than in the other.

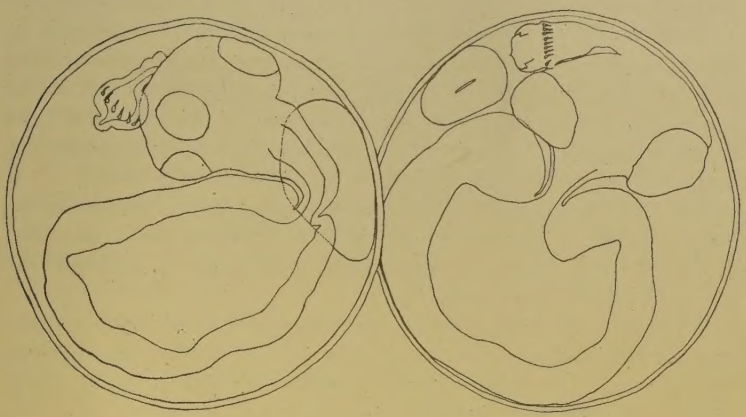

Fig. 3. Two mature cysticercoids (Stage III) from the same fly; with vesicle lying free in cavity of cyst.

## 6. Feeding Experiments.

With such a small amount of material very little could be done in the direction of experimentally infecting a vertebrate host, but the few experiments that were tried are perhaps worth mentioning.

On April 2, 1918, some fresh cysts were introduced by means of a pipette into the gullet of a lizard (*Ophiops elegans* var. *meizolepis*). The animal died on the 6th of the same month, probably as a result of too much exposure to the sun; examination of intestine and faeces revealed nothing.

On April 23 a similar attempt was made to infect two other lizards of the same species, also with negative results.

On April 24 some fresh cysts were introduced into the gullet of a young rabbit. Twelve examinations in the course of 25 days of the

faeces of the rabbit, after shaking up with saline and centrifuging showed no cestode ova or any other sign of infection.

Equally negative results attended the attempt to infect a frog (*Rana esculenta*).

### 7. Literature and Conclusion.

In the literature of the Cestodes I have been able to find mentioned but two species for which the house-fly is claimed as the intermediate host. Both of these occur in chickens; they are: (1) *Choanotaenia infundibuliformis* (Goeze 1782) Raillet 1896, and (2) *Davainea cesticillus*.

With regard to Choanotaenia the account of the development of this species is contained in a much quoted paper of Grassi and Rovelli (4) in which they write, after remarking upon the wide distribution of this species, which they call *Taenia infundibuliformis*—"La ragione si è che l' oste intermedio è la Mosca (*Musca domestica*). Noi abbiamo nella sua cavita addominale trovati varie volta da 2 a 30–35 cisticercoidi, il cui scolice è identico a quello della *T. infundibuliformis*. Nello stesso laboratorio della Universita di Catania, dove tenevamo molto materiale di Tenie dei Polli per i nostri esperimenti, abbiamo potuto accertarci che alcune Mosche si erano infettate."

They give one small figure of the cysticercoid which bears a rough general resemblance to that in my figure 3, but neither the envelope nor the vesicle *V* is shown, and the rostellum appears to be very small or in a very completely retracted condition.

In another paper (3) in which this species is mentioned Grassi and Rovelli merely state that the intermediate host is the fly and that the cysticercoid lacks a "tail," such as occurs in *Dipylidium caninum*, and which is perhaps represented in my species by the vesicle *V*, in Figs. 1 *b* and 1 *c*.

Stiles (6) gives a detailed account of the adult of *Choanotaenia infundibuliformis* under the name of *Drepanidotaenia infundibuliformis* (Goeze 1782), together with a long list of synonyms. On p. 45 he states: "Were it not for the fact that the original host (chickens) is known, I have the most serious doubt whether it would ever be possible to recognise this form; and whether even the numerous specimens recorded from chickens as *T. infundibuliformis* are to be considered as such is, in my opinion an open question." On the same page he also states: "as for the supposed life-history, with the fly as intermediate host, although I am not willing to deny the correctness of the hypothesis, I do insist that it is only an hypothesis...."

Detailed descriptions of the adult of this species will also be found in Ransom (5) and Cohn (2).

As to *Davainea cesticillus*, the second species I have mentioned, J. E. Ackert (1), in a preliminary communication, has described experiments in which he succeeded in infecting young chickens by feeding them on flies that had themselves been fed on the eggs of this tape-worm. He figures the hexacanth embryo but not the cysticercoid.

It will be seen therefore that it is impossible to identify my cestode with either of the above from the stages I have described. At the time my observations were made no literature bearing on the subject was available, and the possibility of the fowl being the definitive host was not considered.

It is hoped in publishing these notes that they will direct attention to the occurrence of this parasite in Mesopotamia and that they may be of assistance to anyone who is in a position to pursue the matter further.

I wish to express my thanks to Professor R. T. Leiper, D.Sc., M.D., for much help and criticism in the preparation of these notes for publication; and to Capt. P. A. Buxton for the use of material found in the course of his own dissections, and for constant assistance in the examination of experimental animals.

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# ON CARRAGEEN. *CHONDRUS CRISPUS.*

By PAUL HAAS and T. G. HILL.

*Botany Department, University College, London.*

(With 5 Text-figures.)

The scarcity of gelatine during the period 1915–1919, and in many cases its poor quality, led those concerned to seek for a substitute, with the result that carrageen once more came back to favour. In this respect Mrs Maitland Malcolm was most active; the Ministry of Food expressed its approval of "Irish moss" in the preparation of invalid foods, and its collection was organised by the Food Production Department. The observations made by various members of the British Red Cross Society have thus led to a revival of interest in the plant, and at the instance of Professor Oliver we undertook its examination. It has been considered desirable to publish this preliminary general account for the use of those concerned not with the chemistry of the substance but with its value as food and in the arts, and to reserve for a future occasion our observations, as yet incomplete, on the more purely scientific aspects of carrageen.

*Chondrus crispus,* Lyngb., is a member of the Gigartinaceae, a family of the Florideae (red seaweeds), and is widely distributed on rocky sea shores. In the British Isles it is abundant on the west coasts of Ireland and Scotland and on the south and north-west coasts of Wales. In England it is reported from the south-west—Dorset, Devonshire, particularly the south coast, Cornwall and Somerset—and from North Yorkshire and Northumberland. It extends from three-quarters tide level to below low water mark and favours sloping localities, especially where the rocky shore gently slopes to low water mark.

*Chondrus crispus* is a perennial plant which reaches its maximum vegetative development in spring and summer. It is characterised by a tufted habit, the fronds, narrow, flat and stalk-like basally, branching distally into flat or curled expansions, varying in colour from purple brown to red purple in more strongly illuminated situations. Iridescence is a characteristic feature. The reproductive organs are immersed in the fronds.

The form of the plant shows much variation in the breadth and branching of the fronds, according to the conditions of growth, exposure to waves and surf for example. Four typical forms are shown in the accompanying figures, which were drawn by Miss Matilda Smith from specimens in the Kew Herbarium.

The "Irish moss" of commerce consists of the dried yellowish brown fronds of *Chondrus crispus* mixed with some *Gigartina mamillosa* (Fig. 5). The weed is collected at low tide and is spread on shingle or grass where

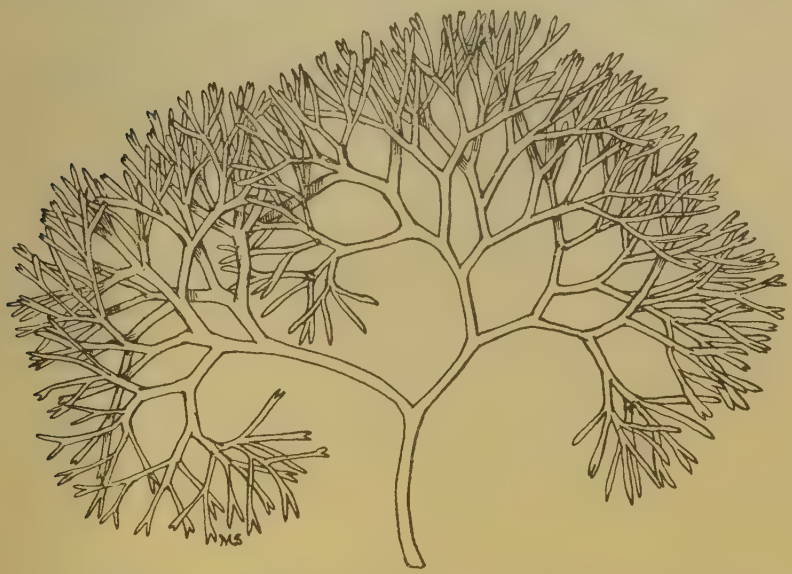

Fig. 1. *Chondrus crispus.* Narrow form from swift channels and rough water. (Nat. size.)

it is cured by watering with either fresh or salt water and bleached and dried by exposure to the sun.

The wholesale price varies from £30–£90 per ton according apparently to the fancy of the dealer.

### *Uses of Irish Moss.*

Irish moss or carrageen when boiled with water swells up and gives a gelatinous mass; on straining this yields a more or less transparent viscous solution which gelatinises on cooling. This material has been used in medicine as a demulcent and has by some been considered to

be a specific for bronchial and other pulmonary complaints. In invalid cookery carrageen has been used in the making of jellies, but in ordinary domestic cookery it has hitherto been rarely used except in places where

Fig. 2. *Chondrus crispus.* Narrow form from pools on open coast or roughish water which is well aerated. (Nat. size.)

it naturally occurs[1]. In pharmacy it is employed as an emulsifying agent for oils, and it is also employed for fining beer, more especially in America; in the arts it is further used as a substitute for size in the

[1] Mrs O'Connell of Clifden, Connemara, informs us that only the better class folk in the west of Ireland use it as a substitute for gelatine while the peasantry do not even know of its value. The statement found in the literature that Irish moss is extensively used in Ireland as a food for human beings is therefore not borne out by this information; on the other hand, according to Mrs O'Connell, carrageen jelly and skim milk yields excellent results in the dietary of calves. See also Harvey, *Phycologia Britannica*, vol. III, London, 1846–51.

manufacture of paper, in the finishing of textiles[1], for thickening pigments and in the making of plaster for walls.

### METHOD OF EXTRACTING THE GELATINISING SUBSTANCE.

The dried weed as received from the collectors was handpicked, foreign algae and other matter being removed; the material was then rapidly rinsed in two changes of distilled water so as to remove adhering dust and sea water salts. The fronds swell much in fresh water and

Fig. 3. *Chondrus crispus.* Medium form from fairly calm water. (Nat. size.)

solution of the gelatinising substance immediately begins; it is therefore essential, if an undue loss is to be avoided, for this preliminary washing to be carried out as rapidly as possible. After rinsing, the plants were squeezed free from excess of water, spread on clean paper and allowed to dry at room temperature and finally in the steam oven. To prepare the water-soluble gelatinising material two methods of extraction were employed:

[1] See *The Textile Recorder*, 1919, p. 404.

(i) *Extraction with hot water.*

The powder was sprinkled on hot distilled water contained in a beaker flask heated on a boiling water bath and thoroughly stirred to prevent the formation of lumps. Sufficient powder was added to make roughly a 1 per cent. solution. After heating for half-an-hour, the contents of the beaker were filtered under pressure first through linen

Fig. 4. *Chondrus crispus.* Broad form grown under quiet conditions on shallow shores, harbours, and muddy bays. (Nat. size.)

and then through filter paper in a Buchner funnel. The residue was repeatedly extracted in this way and the combined filtrates were then poured on to a shallow tinned copper dish and heated over a boiling water bath. When dry the carrageen extract peeled off.

By this method 70–75 per cent. of water-soluble extract may be obtained.

(ii) *Extraction with cold water.*

The powder was stirred into cold distilled water and after the addition of a little toluene was allowed to stand for 12 hours, with occasional stirring; the supernatant solution was then syphoned off, filtered and evaporated; more distilled water was then added to the residue and the process repeated.

Fig. 5. *Gigartina mamillosa.* (Nat. size.)

With a view to comparing successive fractional extracts each portion was evaporated separately.

Exhaustive extraction by means of cold water as above yielded only 18·85 gms. of water-soluble material from 40 gms. of the ground weed in 36 days.

Whether extracted by hot or by cold water, the appearance of the scale carrageen is a clear gelatine-like substance of a pale dull yellow colour, brittle when quite dry, which apparently keeps well for an indefinite time. Although of similar appearance, it has been found that

the scale carrageen extracted with hot and with cold water differs in certain physical characters, notably the rapidity of solution in water, hygroscopic properties, viscosity of their aqueous solutions and the behaviour of such solutions towards Rochelle salt.

*Action of Rochelle salt.* Rochelle salt (sodium potassium tartrate) has a marked effect upon the gelatinising of solutions of carrageen too weak to form a gel under ordinary conditions; thus 5 c.c. of a 1 per cent. solution of a *hot water* extract of carrageen boiled with five drops of a 20 per cent. solution of Rochelle salt sets to a gel on cooling whereas a 1 per cent. solution of a *cold water* extract is not gelatinised by boiling with Rochelle salt.

This indicates that carrageen contains more than one colloidal substance extractable by water, one of which is more soluble in cold water than the other and this has an important bearing on the mode of preparation of the extract, since a cold water extract is deficient in one of the constituents which may be extracted by hot water.

### Gelatinising Power.

The best method of gelatinising scale carrageen is to soak the material first in water: a rapid absorption takes place accompanied by considerable swelling; solution is effected quickly if the vessel be placed in water heated to about 60° C. As compared with gelatine and agar, carrageen occupies an intermediate position. Both gelatine and carrageen dissolve easily in water at relatively low temperatures; agar, on the other hand, requires prolonged heating at about 90° C. The melting point of agar is correspondingly high, while the melting points of gelatine and carrageen are relatively low. There is a marked difference between these last two substances with regard to the sharpness of their melting points: whilst gelatine passes from the solid to the liquid state with some degree of suddenness, carrageen on melting yields a viscous liquid which unlike gelatine remains thick while hot, and this fact makes it hard to say exactly at what temperature the jelly melts. For this reason Hatschek's method, which works well for gelatine, is consequently not altogether suitable for carrageen and the figures given below obtained by this method are therefore only approximate.

The accompanying table gives the approximate melting points obtained for equivalent strengths of gelatine and carrageen (hot extract):

| | Gelatine | Carrageen |
|---|---|---|
| 3 % | 27·7° C. | 27–30° C. |
| 5 % | 29·5° C. | 40–41° C. |

From these figures it will be seen that whereas the melting point of a 3 per cent. solution is approximately the same for both substances, the melting point of a 5 per cent. solution of carrageen is considerably higher than that of a 5 per cent. gelatine, and that, so far as physical properties are concerned, a 5 per cent. carrageen could be used for slope or plate cultures, without fear of liquefaction in incubators at or slightly above blood heat.

The only published observations on the melting points of carrageen which we have been able to trace are those of Standford[1] who compared the gels obtained from various seaweeds with gelatine. He gives the melting point of a 3·2 per cent. solution of gelatine as 15·5° C. and that of a 3·6 per cent. solution of carrageen as 21° C. These figures are markedly different from those recorded above; it is, however, impossible to examine them critically since Standford gives no information regarding his methods.

The gelatinising property of carrageen is not destroyed by boiling; thus a 3 per cent. aqueous solution of carrageen boiled for 3½ hours under a reflux condenser sets on cooling after a few minutes whereas a 3 per cent. gelatine solution similarly treated does not set for several hours.

## HYGROSCOPIC PROPERTIES.

The dried plant and also the scale preparations of the aqueous extract of the plant are markedly hygroscopic. Very varying figures may be obtained according to the atmospheric moisture obtaining at the time of the experiment.

For the air-dried weed figures varying from 2·54–18·2 were obtained. Other authors give varying figures; thus Church[2] found 18·78 per cent. of moisture in the air-dried plant, Jolles[3] 12·36 per cent. of moisture, and in Thorpe's *Dictionary of Applied Chemistry* the value 17·9 per cent. is given.

In order to compare the hygroscopic properties for hot and cold water extracts of the weed determinations were carried out concurrently on these materials with the following results.

| | | |
|---|---|---|
| Cold water extract ... ... | 23 % | moisture |
| Hot water extract of residue ... | 19 % | ,, |
| Direct hot water extract of weed | 21 % | ,, |

[1] Standford, *Pharm. Journ.* 1884, XIV, 1010.
[2] Church, A. H., *Journ. Bot.* 1876, XIV, 71.
[3] Jolles, M. and A., *Just. bot. Jahresber.* 1896, II, 452.

It may be remarked that the accurate determination of hygroscopic moisture is a matter of some little difficulty owing to the fact that the material when dried to constant weight in a steam oven may show signs of darkening and decomposition at or about the final stage.

### Ash Content.

Carrageen contains a relatively high percentage of ash both in the plant and in the scale; in the latter instance the amount varies according to the method of preparation, the earlier fractions of cold water extracts yielding the greatest amounts. The following are typical analyses:

A. The untreated plant gave 14·6 per cent. ash.

B. Scale carrageen:

| | | |
|---|---|---|
| (i) | Cold water extract ... ... ... | 5th fraction gave 27·07 per cent. ash.<br>10th fraction gave 24·32 per cent. ash.<br>30th fraction gave 21·50 per cent. ash. |
| (ii) | Hot water extract of residue remaining after more or less complete extraction with cold water | gave 16·30 per cent. ash. |
| (iii) | Hot water extract. (Plant extracted with hot water only) | gave 22·70 per cent. ash. |
| (iv) | Residue from (iii) ... ... ... | gave 5·19 per cent. ash. |

Church gives 14·15 per cent. of ash in the dry plant, Flückiger and Hanbury[1] found that the aqueous extract of the plant and the plant itself contained more than 15 per cent., Czapek[2] gives 20·6 per cent., Flückiger and Obermaier[3] found 16 per cent., whilst Jolles found but 1·59 per cent. This last result is obviously incorrect, but the mistake may be due to a displacement of the decimal point.

It was found impossible to remove the mineral constituents by dialysis, a fact commented on by previous authors; indeed in one instance an increase of ash on dialysis was found to obtain[4]. For our own part we found that dialysis of a solution of scale carrageen extending over a period of nearly a month in running water reduced the ash from 22·79 per cent. to but 20·55 per cent.

That this reluctance to dialyse was not due to any peculiar attraction of the colloidal material for salts was shown by the fact that a solution of scale carrageen could be entirely freed from added sodium chloride or sulphate by two days' dialysis.

Initial treatment of the solution with dilute hydrochloric acid in the

[1] Flückiger and Hanbury, *Pharmacographia*, London, 1874.

[2] Czapek, *Biochemie d. Pflanzen*, II, 818.

[3] Flückiger and Obermaier, *Schwerz. Wochens. Pharm.* 1868, XIII.

[4] Moeller and Thoms, *Real Enzyklopädie d. gesammten Pharmazie*, 1900, III, 382.

cold or with dilute sodium carbonate followed by filtration and dialysis also failed to have the desired effect.

Subsequently it was found that the amount of sulphate which is precipitated from an aqueous solution of the extract is very much greater after hydrolysis with dilute acids than before such hydrolysis; this observation may account for the difficulty experienced in removing the ash constituents by dialysis.

## NITROGEN CONTENT.

Both the weed and the product obtained from it by extraction with water give positive reactions for proteins with concentrated nitric acid and with Millon's reagent, but the glyoxylic acid reaction for tryptophare is not given by either.

Estimations of nitrogen in the dried weed and the various extracts gave the following figures:

| | | |
|---|---|---|
| A. | Dried plant ... ... ... ... | 1·93 % |
| B. | Scale carrageen: | |
| (i) | Cold water extract ... ... ... | ·99 „ |
| (ii) | Hot water extract of residue from (i) | 1·09 „ |
| (iii) | Direct hot water extract ... ... | ·82 „ |
| (iv) | Residue from (iii) ... ... ... | 4·82 „ |

The figures given by previous authors for the nitrogen content of the plant are: Jolles 2·08 per cent., Flückiger and Obermaier 1 per cent., and Thorpe[1] 1·5 per cent.

## HYDROLYSIS.

The use of carrageen as a food naturally leads to the examination of its behaviour under the action of various hydrolytic agents. The work of others has shown that the water extract of carrageen is a polysaccharide which is converted into various sugars by the action of strong mineral acids[2], but from this it does not follow that carrageen can be hydrolysed by the agents it normally encounters in the human or in the animal system. For this reason many experiments were carried out with solutions of scale carrageen in order to find out the effect of relatively weak hydrolytic agents. In all cases the presence of reducing sugars after the reaction was taken as a positive result: controls always were employed and due precaution taken to guard against bacterial action. Since scale carrageen gives a slight acid reaction, its solution was rendered neutral or alkaline when appropriate.

[1] Thorpe's *Dictionary of Applied Chemistry*.

[2] Our observations on this aspect of the subject are reserved for a future occasion.

*Ptyalin.* Undiluted filtered saliva and diluted saliva, obtained by washing out the mouth with distilled water, gave positive results when incubated at 50° C. for several hours, especially when the medium was strongly alkaline. At body temperature the results after 12 hours were either negative or very feebly positive.

*Extract of pancreas.* Negative results both at 37° C. and 50° C.

*Hydrochloric acid.* A 0·2 per cent. solution of hydrochloric acid was employed so as to have an acidity approximating to that of the gastric juice. A slight positive result was obtained after three hours' incubation at 37° C. In the same period a much stronger reaction obtained after incubation at 50° C.

*Citric acid.* A 5 per cent. solution acting for two hours at 37° C. gave a similar result to that obtained with 0·2 per cent. hydrochloric acid at 50° C. Heated on a boiling water bath for a few minutes with 5 per cent. citric acid carrageen loses its gelatinising power, and the solution acquires a strong reducing action to Fehling solution. The difficulty experienced in making shapes of carrageen flavoured with lemon juice is thus accounted for by the hydrolytic action of the acid.

The above observations suggest that the carbohydrate portion of carrageen is likely to be but slightly affected by the digestive juices of the human body[1] and that the value of carrageen as a food resides in its physical rather than in its chemical properties. The favourable results observed after its use in invalid diet, judging from the published recipes for various dishes, are due to the other ingredients—sugar, milk, eggs, etc.—rather than to the carrageen which provides a pleasing vehicle: moreover when it is borne in mind that the strength of the jelly is rarely likely to exceed 5 per cent., the amount of carrageen consumed in such dishes would be insignificant.

In view of the modern interest in vitamines and the possibility of these substances being responsible for the favourable results recorded in the use of carrageen, Dr Drummond, of University College, kindly undertook the investigation of the material from this point of view; he reported that he was unable to find any traces of either of the fractions associated with vitamines.

[1] The fact that cattle are capable of digesting cellulose by bacterial action in the intestine makes it impossible to express any views as to the digestibility of carrageen by cattle without special experiments.

# FRIT FLY (*OSCINIS FRIT*) IN WINTER WHEAT.

By F. R. PETHERBRIDGE.

(*School of Agriculture, Cambridge.*)

In a previous article[1] the writer has given examples of bad attacks of this fly on winter wheat following leys containing either rye grass or Italian rye grass.

The following experiments show that this is due to the fact that the autumn brood of flies lay their eggs on the grass, and that the larvae feed on the shoots, and after the grass is ploughed in, they eventually migrate to the young wheat plants.

In 1917 a bad attack of frit fly was noticed on one part of a field of wheat on the University Farm, whereas the other part of the field was practically free from attack.

The following shows the differences in the previous treatment of these two pieces of wheat.

Part A. Badly attacked by Frit Fly.

Crop in 1916—*Italian rye grass and red clover.*
Ploughed November 3rd, 1916.
Little Joss sown November 10th, 1916.

Part B. No Frit Fly attack.

Crop in 1916—*Red Clover.*
Ploughed October 10th, 1916.
Little Joss sown October 23rd, 1916.

As a result of this and other observations, an experiment was arranged to see if the ploughing in of the rye grass before autumn would prevent an attack of frit fly.

In 1917 a piece of trefoil and Italian rye grass was divided into three plots.

*Plot A* was ploughed on July 12th,
cross ploughed on July 14th.
Cultivated from August 26th onwards.
Harrowed on September 26th.
Drilled on October 3rd.

[1] *Loc. cit.* vol. iv, Nos. 1 and 2, September 1917.

*Plot B* Fed off by sheep.
Ploughed on October 1st,
then harrowed,
and drilled on October 3rd.

*Plot C* Fed off by sheep.
Ploughed on November 1st,
then harrowed,
and drilled on November 3rd.

The results observed were as follows:

*Plot A.* Although I made several careful searches I did not find a single plant attacked by frit fly.

*Plot B.* Fairly bad attack of frit fly. About 10 per cent. of the plants attacked.

*Plot C.* This plot was damaged by birds, so that it was difficult to estimate the amount of damage, but many frit larvae were found.

This experiment was repeated in 1919–1920 with a view to determining when the frit fly larvae migrate from the rye grass into the wheat.

Before ploughing in the autumn the Italian rye grass was examined and found to be very badly attacked by frit fly larvae, which were feeding on the young shoots. The intensity of the attack was probably increased by the ploughing up of over 90 per cent. of the rye grass in summer and thus leaving a much smaller amount of grass in which the flies could lay eggs.

*Plot A.* Crop in 1919—Italian rye grass and trefoil.
Ploughed July 25th.
Wheat drilled October 30th.

Very few frit larvae found in wheat. A few also found in Italian rye grass plants not ploughed under. A few plants attacked by wheat bulb fly.

*Plot B.* Crop in 1919—Italian rye grass and trefoil.
Ploughed November 7th.
Drilled November 20th.

*About* 25 *per cent. of the plants attacked by frit larvae.* A few plants attacked by wheat bulb fly.

*Plot C.* Crop in 1919—Rye and vetches.
Folded with sheep.
*Wheat drilled in July*, 1919.

A few frit fly larvae found. A few wheat bulb fly larvae attacking wheat and couch (*Agropyrum repens*).

*Plot D.* Crop in 1919—Mangolds and cabbages.
Wheat drilled November 20th.

No frit fly larvae found. Fairly bad attacks of wheat bulb fly in patches.

*Plot E.* Crop in 1919—Mangolds, swedes and turnips.
Wheat drilled December 18th and 19th.

No frit fly larvae found. Fairly bad attacks of wheat bulb fly in patches.

In the above experiments the only loss of crop from frit fly was after Italian rye grass ploughed in during the autumn. The ploughing in of the rye grass before harvest (*i.e.* bastard fallowing) prevented a loss of wheat from the attacks of frit fly.

On Plot B tufts of buried rye grass were dug up during the winter and examined for frit fly larvae at intervals of about a month.

On January 7th a large number of frit fly larvae were present in the rye grass, and many of the shoots were not decayed.

On February 6th a few frit larvae were found to be attacking the wheat plants, but large numbers were still present on the rye grass.

Throughout February and March the attack on the wheat gradually became worse, whereas the number of larvae in the rye grass gradually became fewer. April 7th was the last date on which frit fly larvae were found in the buried rye grass. By this date much of the rye grass was decayed, but a few shoots seemed to be suitable as food for the larvae.

## Pot Experiments.

These experiments were carried out in 5 inch pots in the laboratory.

| No. of Pot | Procedure | Remarks |
|---|---|---|
| 1 | Tuft of rye grass (dug up from Plot B) containing frit fly larvae buried in soil in pot. Wheat sown February 16th | 2 plants attacked on March 16th<br>9 plants attacked on April 17th |
| 2 | 9 frit fly larvae from buried rye grass placed near seedling wheat plants on February 6th | 3 plants attacked on March 26th |
| 3 | Small pieces of rye grass containing 6 frit fly larvae buried near seedling wheat plants on February 2nd | 3 plants attacked on March 26th |
| 4 | 8 frit fly larvae from buried rye grass placed near seedling wheat plants on February 9th | 2 plants attacked on March 3rd |
| 5 | 7 frit fly larvae from buried rye grass placed near seedling wheat plants on February 16th | 3 plants attacked on March 26th |
| 6 | 4 frit fly larvae from buried rye grass placed near seedling wheat plants on February 2nd<br>2 more added on March 3rd<br>1 more added on March 25th | 1 plant attacked on March 26th<br>3 plants attacked on April 1st<br>Examination of the attacked plants showed that the damage in all cases was due to frit fly larvae |

No flies hatched out from these pots. An examination of the attacked plants showed that some of the larvae died before pupation. The single pupa which was found was parasitised.

Of the cases of winter wheat attacked by "frit fly" which I have examined during the last few years, nearly all have been after a ley containing one of the rye grasses ploughed up during the autumn.

On a farm in Norfolk only one field of wheat after rye grass had escaped. This was ploughed up in the middle of August, whereas the fields which were attacked were ploughed up much later.

In Cambridgeshire I found a piece of wheat slightly attacked, following a crop of turnips, but in this case the land was very foul with couch (*Agropyrum repens*) and other grass weeds, so that it is quite probable that the eggs were laid on these.

The only other exception was after a crop of white clover, where it is probable that grasses were present with the clover, although not sown.

I have never seen any appreciable reduction in crop from an attack after potatoes, beans, corn or fallow, but it seems probable that if after either of these the land was covered with grass weeds during the early autumn, the wheat following might be attacked. I have often seen wheat after the above crops free from attacks when neighbouring fields were attacked.

The damage done by the frit maggot seems to be more on a loose tilth than on a compact tilth. In the former case a larger percentage of plants die, probably because they do not tiller so quickly as on firmer soils. Fields in good condition suffer less than those in poor heart probably for a similar reason.

These experiments and observations prove that the frit fly larvae present in the rye grasses are capable of migrating to and damaging wheat plants after the grass is ploughed in.

They also show that an attack of frit fly on winter wheat following rye grass may be avoided by bastard fallowing. Wheat after a bastard fallow, however, is very liable to a bad attack of wheat bulb fly (*Leptohylemyia coarctata*), although in the above experiments this pest did very little damage after this method of procedure.

# SOME REMARKS ON THE METHODS FORMULATED IN A RECENT ARTICLE ON "THE QUANTITATIVE ANALYSIS OF PLANT GROWTH."

By R. A. FISHER,

*Chief Statistician, Rothamsted Experimental Station.*

In the *Annals of Applied Biology* has appeared a paper(1) by Briggs, Kidd and West; according to the authors "The series of articles of which this is the first instalment, constitutes an attempt to formulate methods for the quantitative analysis of plant growth and to apply these methods to data which have been lying dormant in the literature for forty years."

That the paper here criticised is primarily concerned with the methods by which primary observations are to be treated in the study of plant growth is emphasised by a reference made to it in an earlier paper(3) in the *New Phytologist.* "*The Relative Growth Rate, R,* is the weekly percentage rate at which the dry weight increases. It may be assumed for purposes of calculation that the increase from week to week takes place exponentially, $R$ being the exponent, or that it takes place linearly. Both are approximations. As to the relative merits of the two different methods the reader is referred to (4)."

It will be noticed that no definition is here given of the *Relative Growth Rate, R,* except for the case in which the mass increases exponentially, when this is so

$$m = m_0 e^{Rt} \quad \text{.....(I)}$$

where $m$ is the mass and $t$ the time, and $R$ may be calculated from any two observations:

$$R = \frac{\log m_2 - \log m_1}{t_2 - t_1} \quad \text{.....(II),}$$

where the suffixes 1 and 2 indicate the first and second observations.

This is the quantity termed the *Efficiency Index* by Blackman(1), and which may be correctly termed the *Relative Growth Rate*; we shall use the latter term. No indication is given above as to what meaning is to be attached to the term *Relative Growth Rate* when it is assumed that the increase takes place linearly or as to how it is to be calculated from the observations.

It is here fitting to remark that whatever assumptions are made as to the variations of growth rate with time, the relative growth rate *at any instant* is necessarily

$$\frac{1}{m}\frac{dm}{dt} = \frac{d}{dt}\log m \quad \text{.....(III);}$$

the value of $R$, calculated by formula II, therefore gives correctly the average value of the relative growth rate over the period between the two observations, whatever may be the nature of the changes in relative growth rate over this period. This property of formula II, and the precise definition of relative growth rate at any instant given by III should be borne in mind in considering the confused and contradictory use of the term in the ensuing quotations.

For when we refer to the paper under discussion (4) to ascertain what are the two methods of calculation, the merits of which are to be discussed, we find that the term relative growth rate is now applied to a quantity calculated as a schoolboy is taught to calculate Simple Interest. The justification of this procedure must be quoted in full ((4), p. 104).

> There are various methods of presenting the results, and in the first instance we shall use the relative growth rate curve. The principle of the proposed method of expressing rate of growth is analogous to that of the method by which the rate of most reactions, both chemical and physiological, are expressed, namely amount of change per unit of material per unit of time. Since the amount of material in the growing plant is constantly changing, and since the relative rate of growth is not constant, as the following analysis will show, to achieve mathematical accuracy, the increase should be measured over an infinitely short period. This procedure is manifestly impossible, and as we have no exact knowledge of the way in which the relative rate of growth varies, over a given period we have adopted the following purely conventional method of defining relative rate of growth. The relative rate of growth of a plant during any given week of its life-cycle, is the amount of dry matter which 100 gms. of dry matter taken at the beginning of the week adds during the week. A week has been chosen since this is the usual interval between determinations of dry weight in most experiments on growth in plants. It must be noticed that the method does not pretend to mathematical accuracy, being merely an approximate average for the week, but with such results as are at present available nothing more accurate can be obtained."

The most striking feature of this paragraph is the contrast between the precision of the first definition of relative growth rate contrasted with the inconsequent arbitrariness of the method proposed for its calculation. Nothing could be more clear than to define relative growth rate as "amount of change" (*i.e.* increase) "per unit of material per unit of time"; the statement is equivalent to that of expression III. But why, if so precise a definition can be given of the quantity we wish

to measure, should we be reduced to measuring it by a "purely conventional method" which "does not pretend to mathematical accuracy?" The reasons given for this falling away are quite inadequate. It is true that "to achieve mathematical accuracy," in determining the rate of growth at any instant, "the increase should be measured over an infinitely short period"; it should also be measured with infinite accuracy, and on an infinite sample of plants. But the discussion being devoted to the quantitative analysis of such data as Kreusler supplies, it is not clear what would be gained if we could determine the relative growth rate with mathematical accuracy at any one instant. The environmental data quoted by Briggs, Kidd and West are the *Mean Temperatures* for the week, and in some cases the total hours of sunshine. Both of these may be regarded as mean values of quantities which vary in an unknown manner during the week. The corresponding quantity which we require for comparison with them is the mean value of the relative growth rate, which is given with mathematical accuracy by formula II. The difficulty of obtaining the relative rate of increase with high accuracy at any instant thus hardly justifies the complaint that "nothing more accurate can be obtained" than a "purely conventional method," which in fact introduces errors up to 100 per cent. or more!

For the Simple Interest formula employed

$$100 \times \frac{m_2 - m_1}{m_1(t_2 - t_1)} \quad \text{.....(IV)}$$

is extremely inaccurate in relation to the data to which it is applied. The principal cause of this inaccuracy is that the dry mass of the active plant is assumed to have throughout the week that value that is assigned to it at the beginning of the week. It often happens that during the week the mass has more than doubled; in such cases the relative rate of increase is much exaggerated by reckoning the increase on the initial mass. This error has nothing to do with the assumption of linear increase, for if the rate of increase during the week be assumed to be linear, the mean value of the mass for this period will be

$$\tfrac{1}{2}(m_1 + m_2)$$

and the rate of increase reckoned on this mean mass, gives a much better approximation. The formula obtained in this way

$$100 \times \frac{2(m_2 - m_1)}{(m_1 + m_2)(t_2 - t_1)} \quad \text{.....(V)}$$

is compared with that of Briggs, Kidd and West in the following example ((4), p. 107).

| Date of harvest | Mass | IV | V | II |
|---|---|---|---|---|
| June 1st | ·268 gm. | | | |
| | | 108 | 70·4 | 73·5 |
| „ 8th | ·559 | | | |
| | | 91 | 62·7 | 64·8 |
| „ 15th | 1·069 | | | |

Column IV shows the values given by Briggs, Kidd and West, column V the values obtained from the approximate formula V, which would be appropriate for the assumption of linear increase[1], column II gives the true values of formula II; for comparison all are written as percentages. It will be observed that in the first instance IV is 34·5 in excess of the true value, while V is 3·1 in defect, in the second instance IV is 26·2 in excess, and V is 2·1 in defect. The greater part of the error in IV is due to reckoning the increase upon the initial mass.

The errors produced in this way are very irregular; for positive values the rates of increase are exaggerated, for negative values they are made unduly small; so that if in one week the mass is diminished, and in the second it is increased to its former value, the rate of decrease calculated by formula IV will be smaller than the rate of increase which has exactly counterbalanced it. For very small values the formula is approximately accurate. Applied to such data as those presented its irregularities render it most unsuitable for statistical treatment.

But another irregularity is introduced by Briggs, Kidd and West. In the words of a footnote ((4), p. 105), "Where results are not given for a week, we have calculated the increase per 100 gms. for the period, and divided the result by the number of weeks in the period." Such a procedure would indeed be accurate, if the true measure of relative rate of increase had been chosen. Its effect upon the growth rate as estimated by formula IV may best be shown by recalculating the growth rate in the above example, ignoring the value of June 8th. Then for the whole fortnight:

| | | Relative rate of increase per week | | |
|---|---|---|---|---|
| Date of harvest | Mass | IV | V | II |
| June 1st | ·268 gm. | | | |
| | | 149·4 | 59·9 | 69·2 |
| „ 15th | 1·069 | | | |

The average weekly rate of increase for the fortnight as calculated by the principles of Simple Interest is thus much greater than that of either of the weeks composing it. The value given by formula V, though

[1] In the case of linear increase, the relative growth rate necessarily diminishes, having its highest value at the beginning and its lowest at the end of the period. Column IV then shows the value at the beginning, V the value at the centre of the period, and II, in this as in other cases shows the mean value over the whole period.

not so ridiculously discordant, shows that the error of this formula increases rapidly as the time interval is increased, that of formula III alone gives a concordant result, being the mean of the values of the two component weeks.

This example brings out a point in the utility of II which is worth noting. It illustrates the fact that growth rates calculated by II may be expressed at once in any unit of time, irrespective of the intervals employed experimentally. In general it is most suitable to use the day as the unit, and for this purpose the values in column II need only be divided by 7. For the values of IV a dilemma arises; according to the method of correction quoted above, they also should be divided by 7, although this would lead to values inconsistent with any possible daily increases in weight; the only self-consistent method would be to reckon the interest payable daily on the principles of compound interest, and this if performed by the use of logarithms amounts to calculating the value of column II from that of IV, dividing by 7, and then finding the corresponding value of column IV. This process should be in itself of considerable educative value in the study of these different methods of measurement.

These being the facts, it remains to enquire how it was that with Blackman's work before them, and knowing that the efficiency index had been successfully applied (2), the authors of (4) chose to employ so inaccurate a method of calculation. The point is dealt with in the following passage ((4), p. 106).

"It might be suggested that allowance could easily be made for the continuous increase in dry weight during the week by assuming that this takes place at a uniform rate, and consequently that by means of the following logarithmic formula the rate could be determined:

$$\log W - \log W_0 = r,$$

where $W$ is the dry weight at the end of the week, and $W_0$ the dry weight at the beginning of the week.

In curve A, Fig. 1, this allowance has been made. In curve B, the ordinates are relative growth rates calculated by our method, that is, without making allowance for the continuous increase during the week. The curves show similar variations of growth rate from week to week. The more complicated method, however, does not achieve accuracy, as it rests on the assumption that the rate remains constant during the week, an assumption manifestly incorrect since the rate varies from week to week. Both methods are purely conventional and only approximate to accuracy, and nothing definite is to be gained by adopting the more complicated procedure."

At first sight one might judge from this paragraph that the two methods of calculation had been judged to be practically equivalent by an inspection of the diagram referred to, but a glance at that diagram is sufficient to show that this cannot be so, for while the disagreement is

inconsiderable for the periods of slow increase or decrease, at the time of most rapid growth the formula ultimately adopted is approximately double the true value; whence it must be apparent that any quantitative study of the growth rates is materially influenced by the choice of the measure of growth rate. Nor can the difficulty of calculation explain the choice, for with the aid of a table of natural logarithms the correct value is more quickly calculated than any of its approximations; indeed it is surprising that the experience of calculating one series did not bring conviction on this point. One can only explain the choice by the mistaken impression, which is emphasised above, that the use of the logarithmic formula involved the assumption that the relative rate of increase is independent of the time; an assumption which as the authors state, is manifestly incorrect.

This conclusion is confirmed by an apparently disconnected discussion, with which the paper opens, of the general mathematical formulae which have been suggested to represent the growth history of annual plants; among them there is ascribed to Blackman the view that this growth history can be represented by an exponential curve, that is to say, by a constant relative growth rate. Whether this view of the growth history of annual plants has ever been advocated, I am unable to say, but there can be no doubt that in advocating the use of accurate methods for measuring relative growth rate, Blackman (1) is amply justified.

## Summary.

The methods of calculation formulated by Briggs, Kidd and West for the analysis of plant growth are inaccurate, (i) in introducing a large exaggeration when the plant is increasing in mass, (ii) in applying to periods of varying length a method of calculation which thereby becomes self-inconsistent.

The correct measure for the mean value of the relative growth rate over any period, long or short, is that advocated by Blackman under the name of the "efficiency index."

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# THE INFLUENCE OF SOIL FACTORS ON DISEASE RESISTANCE.

By ALBERT HOWARD, C.I.E.

*Imperial Economic Botanist, Pusa, India.*

(With 5 Text-figures.)

## I. Introduction.

One of the features of the modern literature on agricultural science is the number of papers dealing with the diseases of plants in which insects and fungi are concerned. Much less attention, however, has been paid to disease and to the factors on which disease-resistance depends. It is now well understood that the unit species which go to make up the varieties of cultivated crops differ greatly among themselves in resistance to certain parasites and that in some cases at any rate this character can be inherited. Further, it is a matter of common experience that the various diseases only become epidemic where conditions assist the spread of the parasite and at the same time injuriously affect the condition of the crop which is being attacked. Briefly stated, the ravages of any parasite are found to depend on the kind of plant grown and on the conditions under which it is cultivated. We know practically nothing however of the reasons why one unit species is resistant to disease and why others, belonging to the same variety, do not possess this character to any great degree. Further, little has been done to trace the influence of separate soil factors on disease-resistance.

During the last 15 years, opportunities of growing the same crop in India under widely different conditions of soil and climate and of studying the distribution of the crops of this continent have been utilised. At the same time, many observations on the incidence of disease have been made and of the general conditions which appear to precede serious damage by parasites. In some instances, the matter has been carried further and an attempt has been made to ascertain what soil factors are responsible in lowering the natural resistance of a crop to attack. In practically every case, light has been thrown on the

subject by a systematic examination of the root-system[1]. The results obtained are summed up in the present paper. They are admittedly incomplete but are put forward with a definite purpose, namely, to suggest that much more attention should be paid in future investigations of diseases to the general facts of root-development and to the condition of the absorptive areas of the root-system both before the actual advent of the parasite and during the period when the disease is actually established. Up to the present, attention has been paid chiefly to the influence of soil-aeration and soil temperature on disease-resistance.

## II. Soil-Aeration.

The importance of the soil-aeration factor in the growth of crops has been obscured by several causes. In the first place, the chief agricultural experiment stations at which soil problems have been studied have been situated in humid regions where the soil obtains frequent applications of highly oxygenated water in the form of rainfall. In the second place, the discovery of artificial manures has influenced agricultural science just as profoundly as it has revolutionised practice. When most soils are found to respond at once to applications of combined nitrogen, phosphates and potash or of various combinations of these substances and when artificial manures are purchasable in any market place of the country, it is natural to regard such soil deficiencies as due to exhaustion and to find in applications of artificial manures the natural remedy. Under circumstances such as these no stimulus to the study of factors like soil-aeration is likely to occur. When, however, we push over cultivation into the desert and endeavour to make up for defective rainfall by irrigation which often produces impermeable crusts on the surface, the importance of soil-aeration soon becomes manifest[2]. Deprived of regular applications of dissolved oxygen in the form of rainfall, the crop and the soil have to rely on other means of obtaining new supplies of this gas and of getting rid of accumulations of carbon dioxide. Under such circumstances the physical condition of the soil takes on a new meaning and any cause which affects gaseous interchange between the pore spaces and the atmosphere is found to affect the crop immediately. Not only does soil-aeration influence the amount of growth but also the development of the root-system and the resistance of the crop to disease. In some cases defective soil-aeration actually causes disease.

[1] *Agr. Jour. of India*, Special Indian Science Congress Number, 1917, p. 17.

[2] *Bulletins* 52–61, Agr. Research Institute, Pusa, 1915–16.

*The wilt-disease of Java indigo and other monsoon crops.* These wilt-diseases are examples of a definite disease in which parasites have not yet been shown to play a part.

Java indigo (*Indigofera arrecta* Hochst.), the species now generally cultivated in Bihar, frequently suffers from wilt during the late rains. At the beginning of the monsoon, growth is normal but in wet years a change takes place about mid-July in the character of the foliage while the rate of growth slows down. The leaves alter in appearance, assume a yellowish-green, slaty colour, become reduced in size and show extensive longitudinal folding. After this, leaf fall is rapid until only stunted tufts of foliage at the ends of the branches remain. In severe cases, this is followed by the death of the plant, the process taking place slowly, a branch at a time. The external symptoms of wilt suggest extensive root damage which is confirmed by exposing the root-system by means of a Knapsack sprayer. Wilted plants are found to possess very few fine roots and nodules in an active condition. The main tap-root and the laterals are alive and normal but the fine roots are mostly dead or discoloured and the number of absorbing root hairs is exceedingly small. The destruction of the active root-system including the nodules takes place from below upwards. When wilt is well established, the absorbing roots still alive are all in the upper two or three inches of soil. Evidently some factor is in operation which destroys the fine roots in the subsoil and which afterwards affects those towards the surface.

Other investigators have failed to find any parasite responsible for the trouble. No insects could be discovered attacking the fine roots and none of the well-marked appearances of fungoid attack were evident.

The association of extensive root damage with wilt suggested a detailed study of the roots. For this purpose, it was necessary to expose the root-system without damage including the absorbing areas and the nodules. This is easily accomplished in the fine silt-like soils of Bihar by means of a Knapsack sprayer. The range in the root-systems of the various types which make up the indigo crop was found to be as great as that of the above-ground portion of the plant. The mode of branching of the roots closely corresponded with that of the shoot. The type of rooting could always therefore be foretold from the mode of branching. The root-system in this crop is the mirror image of the shoot. Nodular development was found to be most intense at the break of the rains in May and June and to be most pronounced on the roots near the surface. Soil-temperature was found to affect the growth of roots and a distinct resting period ensued during the cold weather of December, January and

early February. Of great interest were the results obtained when actively growing indigo plants were cut back. This was followed in all cases by the death of the fine roots and nodules and before new shoots were formed extensive root regeneration was necessary. The formation of new roots during the monsoon was found to be more rapid if there was a break in the rains after cutting back, and to take place much more readily in the case of surface rooted types than if the root-system was deep. The next step in the investigation was to determine whether wilt is actually caused by the gradual destruction of the fine roots and nodules as seemed probable. Wilt was produced experimentally in the following ways:

(*a*) By the mutilation of the root-system. One example of wilt produced in this manner may be quoted. An indigo plant was partially cut back on June 21st, 1919. On August 5th, the roots were exposed by the Knapsack sprayer and were found normal and healthy in all respects. Before replacing the soil, the fine roots and nodules on the laterals were removed to the depth of one foot but below this point the soil was not disturbed. Wilt rapidly developed and when the entire root-system was again exposed on August 29th very few active roots were found.

(*b*) By deep interculture, during the rains, of indigo sown in lines. Two well-marked cases of this have occurred at Pusa recently. In 1918, Java indigo, sown in double lines with wide spaces between to admit of interculture, speedily lost in vigour and developed much more wilt than the broadcast crop side by side which was not cultivated. In 1919, the experiment was repeated with four types of indigo sown on two different types of soil. The indigo grown in double lines with interculture yielded less crop and developed more wilt than the neighbouring broadcast plots which were not cultivated. The cultivation was found to destroy the lateral roots near the surface on which the plants were dependent at that period of the year.

(*c*) By October and November cultivation of old indigo, dependent for its crude sap on superficial roots. After the rains, the only active roots of an old indigo crop are quite close to the surface. If the land is cultivated these are destroyed and wilt develops. Mulching the surface with straw to preserve the moisture and to prevent these roots drying up as the season changes has the reverse effect.

(*d*) By cutting back young rapidly growing August-sown plants in October, when the reserve materials in the tap-root are insufficient for root regeneration. Cutting back at this period kills the majority of the plants but a few produce wilted shoots.

(*e*) By complete cutting back in the cold weather when the root regeneration of surface-rooted types is difficult probably on account of low soil-temperatures. In December, 1918, 641 healthy well-developed August-sown plants were cut back when over five feet high. The following February, 162 of these were badly wilted, 185 were partly wilted and 294 were normal. A number of root washings were made and in all cases wilt was found to be associated with the practical absence of root regeneration. The plants which developed wilt were those which had their laterals near the surface, the deeper rooted plants producing normal growth. Thus the monsoon results are reversed during the cold weather. In the cold season, the factor which checks root regeneration is apparently low soil-temperature. This affects surface-rooted plants much more than deep-rooted types. The plants were kept under observation till April by which time a remarkable change had taken place. The rise in the soil-temperature in March caused the wilted plants to recover, root regeneration took place and the growth became normal.

(*f*) By waterlogging slowly from below during the rains, by closing the drainage openings of lysimeters. At the beginning of the rains of 1918, indigo was sown in two sets of lysimeters. These were air-tight cemented tanks, 1/1000 of an acre in area, four feet high, built above the ground level and provided with drainage openings which could be closed at will. In one set, alluvial soil exceedingly rich in available phosphate (0·318 per cent.) from the Kalianpur Farm near Cawnpore was used, in the other light Pusa soil was employed. The latter, when analysed by Dyer's method, gives very low figures for available phosphate (0·001 per cent.). In both soils the indigo in the lysimeters with free drainage escaped wilt altogether. When the drainage openings were closed and waterlogging from below took place all the plants were wilted in both Kalianpur and Pusa soil. Wilt in the Kalianpur soil (rich in available phosphate) was much worse than in Pusa soil (low in available phosphate). The growth in Kalianpur soil was much slower than in Pusa soil.

These experiments establish the cause of wilt. The disease results from damage to the fine roots and nodules under circumstances where root regeneration is difficult or impossible.

As has been stated above wilt generally makes its appearance during the latter half of the rainy season. During this period it is often the cause of low yields of indigo. The agency which brings about wilt during this period has been found to be defective soil-aeration caused by the upward rise of the ground water, combined with the destruction of the porosity of the surface soil by heavy rain. This interferes with soil-

aeration and produces root asphyxiation. Soon after the monsoon begins in June, the level of the rivers rise followed by that of the ground water. The movements of the river levels and the general ground water are illustrated by the curves shown in Fig. 1, which represent the state of affairs of the river at Pusa and of one of the wells (about a quarter of a mile distant) for the years 1910 and 1912.

The curves are typical of the subsoil water conditions of North Bihar

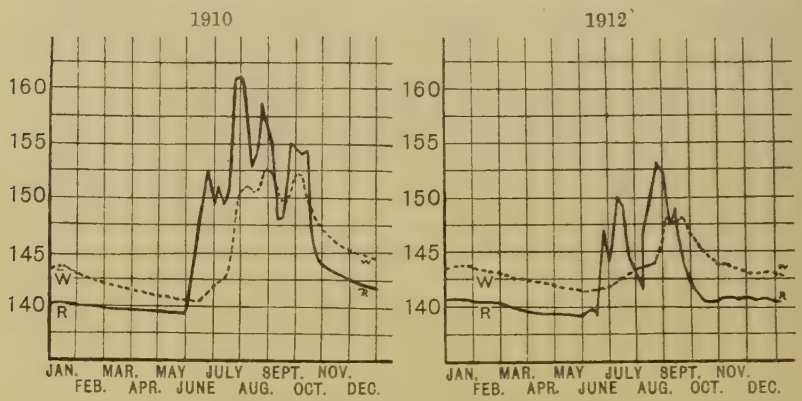



Fig. 1. Changes in the river and well levels at Pusa. The well levels are shown by dotted lines. The observations are expressed in feet above mean sea-level.

Table I. *Percentage of $CO_2$ in the Soil Gas from three different plots in the Botanical Area, Pusa, in* 1919[1].

| Month and date when the soil gas was aspirated and analysed | Plot No. 1, grassed down | Plot No. 2, grassed down but partially aerated by trenches | Plot No. 3, surface cultivated | Rainfall in inches since Jan. 1st, 1919 |
|---|---|---|---|---|
| Jan. 13th, 14th and 17th | 0·444 | 0·312 | 0·269 | Nil |
| Feb. 20th and 21st | 0·472 | 0·320 | 0·253 | 1·30 |
| March 21st and 22nd | 0·427 | 0·223 | 0·197 | 1·33 |
| Apr. 23rd and 24th | 0·454 | 0·262 | 0·203 | 2·69 |
| May 16th and 17th | 0·271 | 0·257 | 0·133 | 3·26 |
| June 17th and 18th | 0·341 | 0·274 | 0·249 | 4·53 |
| July 17th and 18th | 1·540 | 1·090 | 0·304 | 14·61 |
| Aug. 25th and 26th | 1·590 | 0·836 | 0·401 | 23·29 |
| Sept. 19th and 20th | 1·908 | 0·931 | 0·450 | 30·67 |
| Oct. 21st and 22nd | 1·297 | 0·602 | 0·365 | 32·90 |
| Nov. 14th and 15th | 0·853 | 0·456 | 0·261 | 32·90 |

[1] I am indebted to Mr Jatindranath Mukherji for these determinations.

during the rain. That the rise of the subsoil water combined with the consolidation of the surface soil does interfere with soil-aeration is shown by the periodic determination of the soil gases at Pusa during 1919 (Table I), a year when rainfall was below the average and when the movements of the ground water were very slight.

During this year, indigo wilt was almost negligible and only made

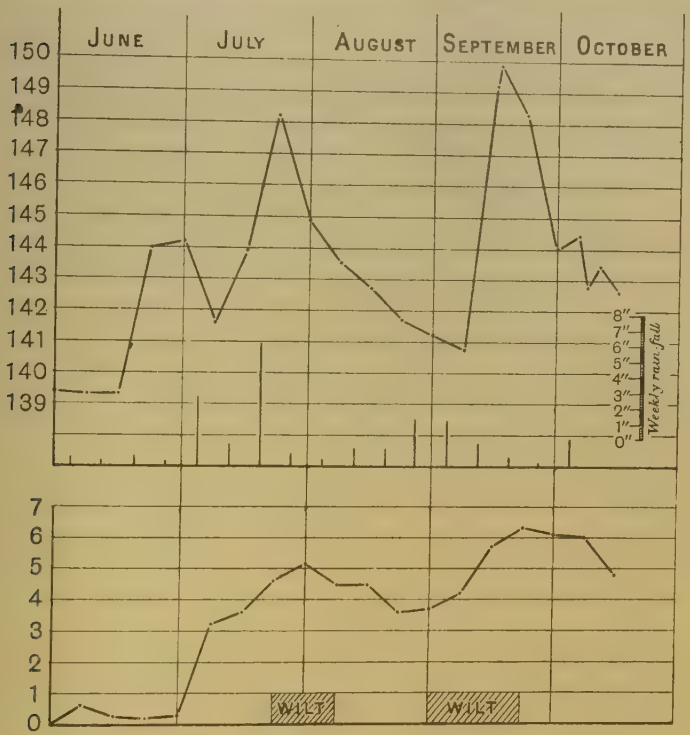



Fig. 2. Rise and fall of the river and well levels at Pusa in 1919.

its appearance at Pusa on two occasions, between July 23rd and Aug. 7th, and again between Sept. 1st and 23rd. A reference to Fig. 2 will show that these attacks followed a rise of the ground water combined with heavy rain. During these periods, the roots of many of the affected plants were exposed and compared with those of normal individuals. In all cases, most of the nodules and fine roots of the wilted plants were dead except a very few near the surface of the ground. The root tips of

normal plants exhibited marked aerotropism and were found to have turned upward towards the air. This continued until a break in the rains and a fall of the ground water restored soil-aeration when normal growth ensued.

Confirmatory evidence of the view that wilt during the rains is due to the destruction of the fine roots and nodules caused by poor soil-aeration has been obtained in several directions. The actual soil conditions under which wilt naturally occurs can be reproduced in a lysimeter by closing the drainage openings. The slow rise of the water-table leads to the destruction of the fine roots and nodules from below upwards and to the production of wilt. Recovery from monsoon wilt takes place in

Fig. 3. The root-system of *Hibiscus Sabdariffa* (left) and *H. cannabinus* (right).

the lysimeters and also in the field after the aeration of the soil improves and when the temperature permits of the regeneration of the roots. Indigo grown on porous soil in other parts of India under a high rainfall, such as Dehra Dun and the Chattisgarh Division of the Central Provinces, escapes wilt altogether. In Bihar, wilt is always most severe in years of heavy rainfall when the subsoil water remains at a high level for long periods. It is negligible in years of short rainfall like 1919 when the rise of the subsoil water is very slight.

Wilt in Bihar during the monsoon is by no means confined to Java indigo. It is common on many deep-rooted varieties of "patwa" (*Hibiscus cannabinus* L.) and "sann" (*Crotalaria juncea* L.) while shallow-rooted types of these two species are little affected. Further,

surface-rooted species like "Roselle" (*Hibiscus Sabdariffa* L.) thrive no matter how wet the monsoon may be. The roots of Roselle in wet years exhibit marked aerotropism leaving the soil and growing all over the surface of the ground. The differences between the distribution of the roots of Roselle and of deep-rooted types of *patwa* are shown in Fig. 3, while in Fig. 4 the roots of an early and late type of *patwa* are illustrated. The surface-rooted Roselle crop and the early types of *patwa* do well at Pusa even if the soil becomes waterlogged occasionally. The deep-rooted types in such seasons, on the other hand, suffer severely from wilt. In such cases, the fine roots are destroyed from below upwards and the details follow closely those already described in the case of Java indigo.

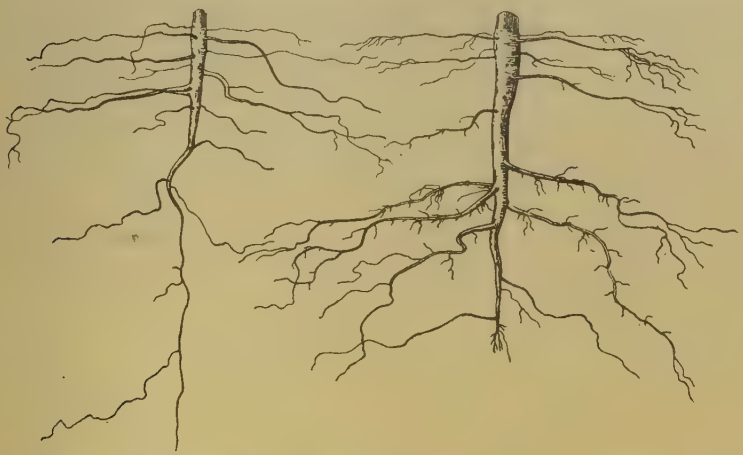


Fig. 4. Early (left) and late (right) types of root-systems in *H. cannabinus*.

Similar results have been obtained at Pusa in the case of two varieties of *sann* hemp (*Crotalaria juncea* L.). The local Bihar variety with surface roots sets seed but the deep-rooted tall variety from the black soils of the Central Provinces suffers from wilt and various insect diseases and hardly yields any seed crop.

The wilt diseases so far dealt with result from the slow destruction of the active root-system which follows the cessation of drainage and aeration during the rains. No parasite appears to be involved in any of these diseases. The remainder of this paper deals with diseases in which either insects or fungi are concerned, but in every case the actual attack follows the operation of some injurious factor such as poor soil-aeration,

a high soil-temperature or increased humidity. All these cases require more detailed investigation which it is the object of this paper to provoke.

In connection with the investigation of the wilt disease of indigo and of *Hibiscus cannabinus*, some observations have been made on the conditions which appear to precede the attacks of two insects *Psylla isitis* Buckt. on indigo and the red cotton bug (*Dysdercus cingulatus* Fabr.) on *Hibiscus cannabinus*.

*Psylla* frequently attacks the stems and leaves of indigo leading to malformation and twisting of the growing points. Sometimes the attack stops at the end of the rains and the new leaves then become quite normal. Stems showing *Psylla* attack below and healthy foliage above are quite common and the attack does not spread to the new growth. It has been frequently observed at Pusa that applications of fresh undecayed organic matter (such as green manure) applied shortly before sowing as well as dressings of oil cake after sowing are always followed by severe attacks of *Psylla*. Examination of the root-system shows restricted and abnormal development and much discolouration of the active roots. The sequence of events is so well marked that the matter deserves to be studied in much greater detail. The fermentation of fresh organic matter in the soil seems to lead to changes in the sap and in the cells of the leaf which predispose the indigo plant to attack. In all cases we have examined, root discolouration precedes and accompanies the insect attack.

Equally interesting are the attacks by the red cotton bug on *Hibiscus cannabinus* at Pusa. These always follow the destruction of the fine roots and the onset of wilt. Year after year *patwa* grows normally during the early rains but when the wilt appears in September and October the plants attract swarms of the red bug. The wilt-free plots of Roselle in the neighbourhood are not attacked. Here again we appear to be confronted with a change in the cell-sap arising from root damage which prepares the way for the parasite.

*The rusts of wheat and linseed.* During the progress of the wheat experiments at Pusa, many hundreds of pure lines have been grown and in many cases the same cultures have been repeated year after year. In some instances, these pure lines have been grown in the field and also in flower-pots. In others, they have been grown immediately after the rains with or without a deep cultivation before sowing. Interesting differences between the amount of damage done by black rust (*Puccinia graminis* Pers.) to the same unit species in the same year have often been observed according to the way the plants were grown. In all cases,

the individuals grown in flower-pots showed much less rust than the same unit species grown in the field and the difference was most marked. In flower-pots the roots of the wheat plants obtain a copious supply of air. This apparently increases the resistance of the plant, the rust colonies remain small and few in number and ripening of the grain takes place normally. When grown in the field, the aeration of the roots is reduced, the rust runs much more rapidly and the grain is often shrivelled. Similar differences in rust resistance are observed between plots of the same unit species when grown on heavy land with or without deep cultivation after the rains before sowing. The better the physical condition of the subsoil, the greater the rust resistance. This matter is being followed up further and the connection between rust attacks and the distribution and character of the root-system is being investigated at Pusa. Some interesting facts have already emerged. Several of the most rust-resistant wheats at Pusa are very shallow-rooted, some of the most rust-liable types from the black soils are exceedingly deep-rooted. Soil-aeration appears therefore to play an important part in the relations between the host and the parasite in rust attacks.

In the case of the linseed crop, the matter is being carried further. A large collection of the linseeds of India has been made at Pusa and the various unit species have been isolated and classified. The unit species fall into three groups according to the size of the seed and the character of the root-system. The linseeds of the black soils of Central India possess large seeds and a deep root-system which enables the crop to withstand the cracking of these soils. The types found in the plains have small seeds and a shallow root-system (Fig. 5). The third group is intermediate in all respects. When grown at Pusa in alluvial soil there is a great difference in the appearance of these groups of linseed. The small-seeded class is very luxuriant and does not suffer from rust and other diseases. The large-seeded deep-rooted class grows and sets seeds with difficulty. The plants appear starved and are often attacked by rust (*Melampsora Lini* Desm.). There is a very great difference in the appearance of the active roots of the deep-rooted types of linseed attacked by rust and the shallow-rooted types which escape this disease. The former appear starved and there is extensive discolouration. The latter are turgid, white and exceedingly vigorous.

*Red rot of sugar-cane.* One of the difficulties in sugar-cane cultivation on the black soil areas of the eastern tracts of the Central Provinces is the prevalence of red rot (*Colletotrichum falcatum* Went.), which, as is well-known, attacks the cane during the ripening period and leads to

a great loss of sugar. This disease is also important in other parts of India, such as North Bihar, the Godavery delta in Madras, where soil-aeration difficulties are common. Some interesting results have been obtained by Clouston[1] in the Central Provinces on the effect of the physical texture of the soil on the resistance of the sugar-cane to this fungus. On the stiff black soils, in this track, red rot is common; on the neighbouring open porous *bhata* soils, however, crops as high as 40 tons of stripped cane to the acre are grown and there is a remarkable absence

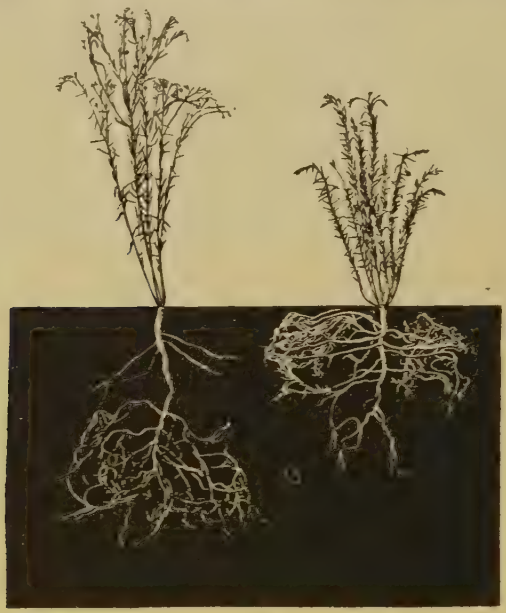


Fig. 5. Linseed from Central India (left) and the Indo-Gangetic alluvium (right).

of the red rot fungus. There appears to be here a case well worthy of more detailed investigation. Soil-aeration and healthy root-development confer a high degree of immunity on the sugar-cane, while the poor physical texture of the black soils appears to render this crop exceedingly susceptible to red rot. The sugar-cane readily lends itself to the investigation of its root-system and of its juices, as the plant is large and abundant material for research is readily available at many centres.

*The protection of fruit trees from green-fly.* The usual method of con-

[1] *Agr. Journ. of India*, Special Indian Science Congress Number, 1918, p. 89.

trolling attacks of green-fly is to spray the trees with some wash in which soft soap is one of the chief constituents. In practice, however, this procedure is of limited value as the affected trees soon become re-infected and the process has to be repeated, an important matter now that labour is becoming so expensive. It is generally considered that the only cause of green-fly attacks is the insect itself. If this is so, there seems no reason why the pest should not spread rapidly in any garden after its first appearance in the spring. This was not the case in the Quetta fruit experiment station, where this pest has been under observation for some years. Frequently trees remained quite free from green-fly in the immediate neighbourhood of others badly affected, and this has happened year after year. It would appear, therefore, that something else besides the green-fly is necessary for successful infection to take place.

Some light has been thrown on the conditions necessary for green-fly attacks as the result of a number of irrigation experiments at Quetta. Following American experience on certain soils, an attempt was made to store up water in the subsoil during the winter and spring for use during the subsequent hot weather, when water is very scarce. The experiments were successful as far as the saving of summer watering was concerned but the system had to be given up on account of the rapid increase in green-fly attacks which accompanied the winter irrigation. After the summer, only one watering is now given in October so as to ensure a sufficient supply of moisture in the soil to prevent the freezing of the roots by early frosts before the winter rains begin. The cessation of winter watering has at once been followed by the recovery of the trees from green-fly attack.

Among the experiments which have been conducted on this subject, the following may be quoted:

1. Four heavy winter irrigations were applied to three plots of peaches and one of nectarines during the winter of 1915–16. In all cases, the trees were very badly attacked by green-fly during April, 1916, and the attack was much more severe than in the neighbouring gardens. Further watering was then stopped and the soil round the trees was broken up down to the upper roots. In this way aeration was restored, and after about a month the new growth produced was free from *Aphides*. The trees then presented a remarkable appearance. The first-formed leaves on each twig showed extensive damage by *Aphides*, the late formed leaves on the same branch were normal and perfectly healthy.

2. The above four plots were treated in quite a different manner during the winter of 1916–17. After the summer, only one watering

was given—on Sept. 30th—and during the winter and spring no irrigation water at all was applied. As a result these plots were practically free from green-fly, the trees grew vigorously and the foliage showed all the characteristic appearances of healthy peach trees. These plots stood out in marked contrast to many of the trees to be seen in the Civil Station when in 1917 the ravages of green-fly were far above the average.

3. Three lines of almonds (a deep-rooted tree), and a plot composed largely of various stocks including plums, almonds and peaches, which were clean cultivated in 1916 and which were remarkably healthy and quite free from green-fly, were sown with shaftal (*Trifolium resupinatum*) right up to the stems in August and September, 1916. Several waterings were applied to these trees during the winter and spring. All the almonds, the seedling peaches, and some of the plum stocks became badly affected by green-fly soon after the leaves appeared at the end of March, 1917. By May, the attack was severe and practically all the young growth was affected. In this case, trees free from green-fly in 1916 lost in a single season all their immunity as a result of winter watering.

4. A number of almond and peach trees—grown under a system of furrow irrigation by which over-watering is almost impossible—were planted in the autumn of 1916 close to one of the lines of almonds which was over-watered during the winter. The object of this was to obtain another demonstration of the fact that insects like *Aphides* are unable to attack healthy plants. The over-watered trees were all affected by green-fly which in no case spread to the plants which had been watered by furrows and which had obtained an abundant supply of air for their roots.

These results, which have been repeated on several occasions at Quetta, suggest that the control of green-fly must be sought elsewhere than in the destruction of the insect. Soil-texture and soil-aeration in Baluchistan are markedly improved by a winter fallow and are known to suffer from over-watering during the cold season. This appears to affect the development of the roots of fruit trees in the spring by interfering with soil-aeration. A change in the sap seems to result after which the trees become attacked by green-fly. The connection between winter irrigation and green-fly has been obtained so frequently and is so definite that more detailed investigations of the soil, of the root-system and of the sap of the trees affected by green-fly is urgently called for. If, as appears possible, it is found that the insect can only attack trees in an abnormal condition, the prospects of the efficient control of this pest becomes much more hopeful.

### III. SOIL-TEMPERATURE.

Although soil-temperature is such an important factor in the distribution of the crops of India, both as regards season and the areas in which they occur, nevertheless but little attention has been paid to this growth factor in considering the incidence of disease. In India, the higher limit of temperature most frequently affects growth and in studying the crops of cold countries like wheat, which can only just be grown in India, this is one of the factors which frequently deserves attention.

*White ants and wheat seedlings.* One of the difficulties in wheat cultivation in Bihar and the eastern districts of the United Provinces is to establish the crop. If sown a few days too early, the seed germinates but the seedlings are rapidly destroyed by *Termites*, whole fields disappearing in a few days. The trouble became of some importance a few years ago in Bihar as it interfered with the raising of seed of the new Pusa wheats on some of the private seed farms[1]. The disease was particularly serious in years when the rains ceased early and when the last monsoon showers of early October, known locally as the *Lathia*, were not received. In such seasons, the advent of the cold weather is always postponed and the cool westerly breezes which normally set in about the middle of October are delayed till nearly the end of the month. The sowing time for wheat in Bihar in years when there is a good *Lathia* is just after the middle of the month and no trouble with white ants need then be feared. When, however, these sowing rains fail, nearly all the fields are destroyed by *Termites*. More damage occurs on low-lying damp heavy soil than on the higher and dryer areas. Examination of the root-system during the attack shows extensive discolouration of the new primary roots and of the first internode. Only in rare cases is there any formation of the secondary system. Before tillering can take place, the first internode is devoured by the white ants and the plants wither. A possible explanation of the trouble appeared to be a high soil-temperature which subsequent investigation seemed to confirm. In several seasons when the late rains failed, a comparison was made between sowings on Oct. 15th and others twelve days later. In addition to the delay, the furrows in the second case were left open for two or three days so as to cool the soil by evaporation. The early sowings were in every case destroyed by *Termites*, while in the later ones the damage was negligible and normal root-development and growth took place.

[1] *Agr. Journ. of India*, XI, p. 351.

The simplest explanation of these results appeared to be a fall in the soil-temperature during the second half of October. That such a fall actually does take place is proved by an examination of a set of soil-temperature determinations made by Leake at Pemberandah in Bihar in 1903–4. In the year in which Leake's readings were taken, the *Lathia* amounted to 3·4 inches of rain and the daily soil-temperature at 4 inches at 1–2 p.m., fell gradually from 29·5° C. on Oct. 16th to 22° C. at the end of the month. This disease of wheat seedlings, which is very common in north-east India, is of some general interest, as the *Termites*, although the apparent cause of the trouble, were in reality engaged in the consumption of a moribund set of seedlings which had been practically destroyed, apparently by a soil-temperature above the maximum for growth. Examination of the root-system in this case provided the clue which soon led to the discovery of the cause of the trouble and to the working out of a simple remedy, which has since been widely adopted on the indigo estates of this tract.

*The rust-resistance of einkorn*[1]. Einkorn (*Tritrium monococcum vulgare* Kche.) is well known to be exceedingly resistant to the attacks of black rust (*Puccinea graminis* Pers.). In 1907, a plot of this wheat was grown at Pusa when it was found to be immune to all the three species of rust which occur in north-east India. The plants however were still in the vegetative condition at harvest time and were allowed to grow during the hot weather to see if any ears would form. No change of this kind took place but early in May they were found to be severely attacked by black rust. Here a prolonged rise of temperature led to the complete loss of disease-resistance in a species considered to be immune to this fungus. Unfortunately, the root-system at the time of the attack was not examined, as the observations were made some years before any attention was paid to such matters at Pusa.

The various diseases referred to in this paper are considered to establish a case for the detailed investigation of the root-systems of plants, combined with a consideration of the chief soil factors, in connection with the study of disease. There seems to be no doubt that the conditions of the active roots profoundly affects the resistance of the plant to the attacks of parasites. What this actually means in the processes of metabolism is a matter for further investigation. The discolouration and damage to the absorbing areas of the root are not unlikely to lead to the entry of substances into the crude sap which

[1] *Journ. of Agr. Science*, II, p. 278.

may entirely alter its value to the plant. This in turn would influence the cell-sap throughout the shoot-system. How such alterations affect the struggle between the protoplasm, on the one hand, and the hyphae of an invading fungus on the other and why insects like *Aphides* thrive on the juices of a Quetta almond tree grown in soil consolidated by over-irrigation the previous winter and disregard it altogether under a different system of soil management are interesting problems for the vegetable pathologist eager to break new ground and to carry his science beyond the beaten track.

The examples quoted suggest another direction in which a knowledge of the root-system is desirable, namely, in the determination of the factors on which the disease-resistance of a unit species depends. A collection of unit species grown under any particular set of soil conditions generally exhibit among themselves marked differences in disease-resistance. At Pusa, it has been found in several crops that an investigation of the root-system throws a considerable amount of light on this point. Both in the rains and in the cold weather, deep-rooted varieties yield less and are more liable to disease than shallow-rooted types. Soil-aeration and its consequences will probably be found to be an important factor in this case. Many more examples of disease-resistance in other parts of the world must, however, be examined before we can say how far immunity depends on morphological root-fitness for the environment and how far it is inherent in the natural resistance of the protoplasm to the invasion of a parasite.

# THE PLANT AS AN INDEX OF SMOKE POLLUTION.

By ARTHUR G. RUSTON, B.A., B.Sc. (London), D.Sc. (Leeds).

*Lecturer in Farm Economics, The University, Leeds.*

(With Plates XXIV and XXV.)

In an earlier communication[1] an account was given of investigations carried out as to the nature and extent of atmospheric pollution by coal smoke in and near an industrial town. These investigations were at first limited to the area within a three mile radius of the centre of Leeds. Later, subsidised by a Government Grant from the Development Fund, they were extended to cover a wider area.

The information thus obtained, as to the relative freedom or otherwise of the various districts from pollution by coal smoke has been exceedingly valuable in studying not only the effects of that pollution on plant life, but also the ways in which the plant itself may be used as an index of the amount of that pollution.

1. In the first place the *General Type of Vegetation* in the smoke-infested areas is instructive in drawing broad conclusions as to the amount of smoke pollution.

Certain plants have been found to be resistant, and others particularly susceptible to smoke damage; and the presence or absence of some of these "Test Plants" is certainly suggestive.

The evergreens, which have to withstand the winter smoke are specially susceptible. Many of these in a smoke-infested area are quickly killed; others first become deciduous, and then finally disappear. Among the evergreens, the privet is particularly interesting as can be seen from the notes summarised in the following table.

| District | Annual deposit of soot in tons per sq. mile | Observations with regard to the privet |
|---|---|---|
| 3 miles to the north of Leeds | 29 | Evergreen, and flowers |
| 2 „ „ „ | 42 | Evergreen, but does not flower |
| 1 mile „ „ | 114 | Few leaves only retained through winter |
| Centre of the city ... ... | 243 | Leaves fall in January |
| Industrial Leeds ... ... | 539 | Leaves fall in November |

[1] Crowther and Ruston, *Journal of Agricultural Science*, vol. IV, Part 1.

In Park Square, near the centre of the city, the laurel, aucuba and rhododendron are deciduous; while in Hunslet, the heart of industrial Leeds, even the box becomes deciduous, and the laurel is killed off in about two years.

Of the evergreens, the most susceptible are the conifers. These trees are xerophytic; their natural habitat is in a pure atmosphere at a high altitude, where the winds are high and the barometric pressure low, and where consequently evaporation from the leaf surface will be great. The soil, however, will be shallow and the roots none too plentifully supplied with moisture; hence there will be a constant necessity to husband the water supply as far as possible. This end has been effected in the evolution of the plant by the development of small leaf surface, and sunk stomata. Both of these characteristics, while useful in the natural habitat of the conifers, prove their undoing in a smoke-infested area. Conifers planted in Hunslet I have seen killed off in less than three months. Twelve years ago, more than twenty conifers were planted in the grounds of the Leeds University, one mile north from the centre of the city. At the end of three years not one was alive. The only two localities in Leeds, in which conifers attain even a moderate growth, are Roundhay and Weetwood, which the earlier investigations showed to possess the least smoke-infested atmosphere.

It is doubtful if they would ever do well in any district, where the annual deposit of soot amounts to more than 50 tons per square mile.

Plants whose leaves are possessed of a crinkled hairy surface, thus easily catching the soot, and of a thin cuticle readily damaged by the acid rain, are also particularly sensitive to smoke pollution. On the other hand, those possessing a hard smooth leathery type of leaf with thick epidermis, like many of the alpines, pinks, carnations, auriculas, London pride, and iris, are particularly resistant.

Thus the primrose does badly in Hunslet, makes little or no growth, rarely flowers, and never lives through more than one winter; while the auricula is as hardy as possible, and not only lives but thrives, grows and spreads. One border five yards long, which last spring was a mass of bloom, is the result of five years' propagation from four small plants. The geranium can be grown in a smoke-infested area, but the calceolaria is always a failure.

Perhaps no plant of this type gives a better index of the amount of smoke pollution than the hollyhock. Six years ago, eight hollyhocks (all propagated from the same parent plant) were planted in tubs containing soil taken from the same field of the Experimental Farm of the University

of Leeds and the Yorkshire Council for Agricultural Education. The tubs were then placed, two at each station, in four different parts of Leeds. At only one station, that furthest north, did they flower the first year. In the second year more than eight feet of growth was made at Adel three miles to the north, while in the industrial area of Hunslet one plant only survived, a wizened, flowerless specimen, nine inches high.

Plants which have to "lay up a store for another year" tell their own tale of smoke pollution. Heavily handicapped in their growth by the effects of these atmospheric impurities they have little chance of laying up reserves. This is noticed both in the case of the bulbous and seed-bearing plants. Radishes will grow in Hunslet, but they will run to leaf and will not form bulbs. Tulips, narcissi, daffodils, scillas and hyacinths can be grown there and they will flower *one* year, but if a second year's bloom is desired, the bulbs must be replaced. Wheat, oats and barley can be grown in Hunslet, and you will get plants, but the grain, as can be seen from Fig. 1, Pl. XXIV, will not be worth the harvesting. You may grow lettuces and cabbages in Hunslet, but you must not expect them to heart.

A casual glance at the hedgerows will give some guide as to the extent of smoke pollution in any particular area, for the hawthorn is one of the smoke-sensitive plants and one of the first to go under.

Notice the flora of the lawns and fields of any particular district. In a smoke-infested area, the leguminous plants, the clovers and vetches will be conspicuous mainly by their absence. The finer grasses, particularly the fescues, will be missing. Coarse growing grasses like bent, couch and Yorkshire fog will be in the ascendant, and acid loving plants like dock, sorrel and plantain will abound. If a lawn is required in the industrial area of Leeds it must be sown down each year.

In the Hunslet Parish churchyard, surrounded on all sides by huge chimneys belching out smoke, even bent and the hardy *Poa annua* have been killed, and the only sign of vegetation is to be found in a few straggling blades of twitch still struggling for existence. The garden of a large residential house left stranded close to the church is a pitiable sight. Its only flower is the iris which still blooms freely, its only shrub or tree is the elder; its only fruit or vegetable is the rhubarb, and its only grass is twitch.

Though, apparently the elder will grow anywhere, no matter how great the pollution from coal smoke, it can still give some indication as to the amount of that pollution. Though it will grow in the industrial

area of Hunslet, it will not flower. Though it will flower in the residential area of Headingley, two miles from the centre of Leeds, it will not fruit. Apparently it will not flower in localities where the annual deposit of soot is more than 200 tons per square mile, nor will it fruit if the annual deposit is greater than 50 tons per square mile.

Evidently the "General Type of Vegetation" in any one particular district will give some information as to the amount of pollution by coal smoke within the area.

2. The *General Appearance of Individual Plants* is also worthy of attention.

No one coming into Leeds by the Great Northern Railway and looking out of the carriage window near Ardsley station could doubt the fact that he was entering a smoke-infested area; for here almost every tree and every hedge has been killed. In the woods of Middleton and Templenewsam, the trees are to a great extent an index of the smoke pollution from colliery, coke oven and brickworks. Here many of the trees, particularly the ash, oak, elm and beech have been absolutely killed, most of them dying from the top. The main drift of the smoke from the industrial area of Hunslet can be easily traced almost to Garforth by the line of dead and dying trees.

Signs of smoke damage to trees can be discerned quite early in the year, in the appearance of the young shoots and buds, many of which are killed off before opening.

The leaf perhaps is the safest index. Before May is out, many leaves in smoky districts are showing characteristic brown blotches. In the case of leaves possessing a thin epidermis like the lime and sycamore, the signs of smoke damage are seen as in the case of Figs. 2 and 3, Pl. XXIV, all over the leaf, wherever the acid rain drops. In the case of the ribes (Fig. 4, Pl. XXV), the leaves of which possess a harder cuticle and have a tendency to offer a convex surface, the damage is shown by a red rim all round the edge. In the case of leaves like those of the laurel and aucuba which possess a hard cuticle and have a tendency to hang downwards, the damage is shown first at the tip of the leaf.

Evidence as to the amount of smoke pollution can also be obtained by the appearance of trees in the autumn; the greater the pollution the earlier the "leaf fall." Ash trees in the purer parts of Leeds often retain their leaves six or eight weeks longer than those in the more contaminated districts. Thus last year in Hunslet with an annual solid deposit of 539 tons per square mile, all the ash trees which remained alive had shed their leaves before the 18th of September, while at Roundhay with an

annual solid deposit of 26 tons per square mile and no measurable acid deposit, practically all were in full leaf up to the beginning of November.

Evidence of smoke pollution is available at any time in the stunted growth of trees and shrubs, particularly of evergreens. The following particulars with reference to the maximum growth of aucubas in different parts of Leeds are striking.

| District | Position | Annual deposit in tons per sq. mile: Total solid deposit | Annual deposit in tons per sq. mile: Sulphur compounds expressed as $SO_3$ | Maximum height of aucuba found in district |
|---|---|---|---|---|
| Weetwood Lane | 3 miles to north | 42 | 28 | 10 ft. 6 in. |
| Headingley ... | 2 „ „ | 78 | 33 | 7 ft. 2 in. |
| University ... | 1 mile „ | 114 | 38 | 5 ft. 8 in. |
| Park Square ... | Centre of city | 243 | 56 | 3 ft. 2 in. |
| Hunslet ... | Industrial area | 539 | 96 | 2 ft. 0 in. |

Differences of the same order have been found in the case of the rhododendron and laurel.

Even the colour of the flowers in the gardens and woods will give some indication of the amount of smoke pollution. As a general rule the greater the pollution the paler the tint, and the more blues and reds will tend to run to white, and the bronzes to yellow. Intensely coloured flowers will show patches, the scarlet of the geranium in a smoke-infested district will be streaked with purple running to blue and even to white. The blood-red wallflower will be broadly streaked with yellow, and in a year or two most of the red will have disappeared.

Certainly the colour of the leaves will be more than suggestive, and signs of smoke pollution are to be found in the black deposit of soot and tar upon the leaf, and the absence of autumn tints.

3. Though, as has been seen, the "General Type of Vegetation" in a district and the "General Appearance of Individual Plants" will give much information as to the amount of smoke pollution in that district, most can be learnt by the "*Detailed Analysis*" *of the plant*. We may notice in a general way that the leaves of plants grown in the town are dirty. This can be readily seen from the photograph of the holly leaf grown in industrial Leeds (Fig. 5, Pl. XXV) and kindly placed at my disposal by Professor Cohen. The upper part of the leaf in question had first been cleaned, before dipping it in boiling water and extracting the chlorophyll with alcohol. We can, however, get a better idea of their relative cleanness or otherwise, by actually estimating the amount of solid deposit upon a unit area of these leaves. This has been done in

the case of a large variety of leaves, and the deposit thus found can easily be correlated with the known amount of smoke pollution in the district in which they have been grown.

The figures given are those found in the case of laurel leaves grown in the districts indicated.

| District | Annual deposit of soot in tons per sq. mile | Deposit on leaf expressed in mgms. per sq. metre of leaf |
|---|---|---|
| Sutton | In the country free from smoke pollution | 0 |
| Weetwood Lane | 42 | 158 |
| University | 114 | 718 |
| Hunslet | 539 | 1620 |

A microscopic examination of the leaf will reveal the fact that when the plants have been grown in a smoke-polluted atmosphere more or less of the stomatal openings will be choked with a tarry deposit. This will be particularly noticeable in the case of evergreens and most especially in the case of the conifers (Fig. 6, Pl. XXV). The leaves from a juniper grown at Garforth, six miles from Leeds, but well in the drift of the smoke from Hunslet, were examined by Mr Hector, Lecturer in Agricultural Botany, and he reported that 75 per cent. of the stomatal openings were more or less choked in this way.

We may notice in a general way that flowers grown in a smoke-polluted atmosphere lose their brightness of tint; but it is possible by means of a tintometer to get an accurate measure of their colour. I am indebted to Mr Frank of the Dyeing Department of the University of Leeds for the trouble he has taken in analysing by means of Lovibond's tintometer the colours of a large number of flowers. These flowers have been grown in each case from plants propagated at the Stapleton Gardens, Pontefract, from the same parent plant, by Mr Dobson, an old student of the Agricultural Department of the Leeds University, whose assistance in this and many other respects has been invaluable.

Three sets of readings are given.

*Blood-red Wallflowers*, June 13th, 1914.

| District | Annual deposit | Direct tintometer readings: Red | Blue | Yellow | Total colour units |
|---|---|---|---|---|---|
| Weetwood Lane | 42 | 36·5 | 7·0 | 4 | 47·5 |
| University | 114 | 22·5 | 2·5 | 9 | 34·0 |
| Hunslet | 539 | 13·0 | 1·1 | 9 | 23·1 |

*Pyrethrum*, June 28th, 1914.

| District | Annual deposit | Direct tintometer readings | | | |
|---|---|---|---|---|---|
| | | Red | Blue | Yellow | Total colour units |
| Weetwood Lane | 42 | 38·6 | 3·8 | 0 | 42·4 |
| University | 114 | 30·0 | 2·8 | 0 | 32·8 |
| Hunslet | 539 | 26·0 | 1·5 | 0 | 27·5 |

*Lupins*, June 18th, 1918.

| District | Growth | Direct tintometer readings | | | |
|---|---|---|---|---|---|
| | | Red | Blue | Yellow | Total colour readings |
| Hunslet | 1 year | 10·0 | 10·2 | 0 | 20·2 |
| „ | 2 years | 7·6 | 5·0 | 0 | 12·6 |
| „ | 3 years | 4·4 | 3·2 | 0 | 7·6 |

The first one shows the tendency of all bronze flowers in a smoke-infested district to run to yellow; the second shows the cutting down of the red and blue tints and the third illustrates the fact that the longer a plant remains in a smoky atmosphere the more it loses the power of producing colour; evidently it is not simply a case of mere bleaching, but a radical change in the constitution of the plant.

We may notice casually the fact that smoke pollution means stunted growth, but we may get a measure both in the laboratory and *in situ* of the growth of plants, where the conditions other than atmospheric conditions are the same; and the relative growth will be found to be roughly inversely proportional to the smoke pollution. In the laboratory the relative growth has been measured by estimating the amount of carbon dioxide assimilated by a unit area of leaf in a unit of time. The following results refer to experiments made with laurel leaves of the current year's growth taken from shrubs grown in the districts mentioned.

| | Annual deposit in tons per sq. mile | Relative assimilation of $CO_2$ per unit area of leaf in unit of time |
|---|---|---|
| Weetwood Lane | 42 | 100 |
| Headingley | 78 | 53 |
| Clarendon Road | 200 | 15 |
| Park Square | 243 | 12 |

*In situ* the relative growth of plants in different districts has been compared, by filling large wooden buckets with soil taken from the same field in the country, sinking the buckets in gardens in different parts of

the town, where the amount of smoke pollution has previously been determined; using the soil for growing a succession of crops each successive year and carefully weighing the produce. In selecting the various stations care was taken that the altitude, exposure and all other conditions other than atmospheric smoke pollution should be as nearly as possible identical. Apart from a few slight irregularities the results indicate a fairly close correlation between the relative degree of purity of the atmosphere in the neighbourhood of the station, as determined from earlier observations, and the actual amount of plant growth obtainable.

| Station | Relative purity of air, as measured by freedom from sulphur | Relative weight of crop | | | |
|---|---|---|---|---|---|
| | | 1st crop Radishes 1911 | 2nd crop Lettuce 1912 | 3rd crop Cabbage 1913 | 4th crop Wallflower 1914 |
| Weetwood Lane | 100 | 100 | 100 | 100 | 100 |
| Headingley | 70 | 90 | 86 | 122 | 32 |
| University | 55 | 60 | 74 | 89 | 6 |
| Park Square | 37 | 49 | 40 | 37 | 2 |
| Hunslet | 34 | 46 | 31 | 15 | 3 |

An examination of the roots of plants will give some indication of the amount of smoke pollution; plants grown in soil that has been for long exposed to such pollution being marked by an almost entire absence of root hairs and fibrous roots. The differences in the root development of the wallflowers grown in soil which had been exposed in the various districts four years were most marked. Oats and barley were the following year grown in the same districts in the native soils and the differences in root development were still more marked.

An examination of a felled tree will often give valuable information as to the purity of the atmosphere in which it was grown. The tree automatically keeps a record of its yearly growth, and the presence of any inhibiting factor will make itself known by the narrowing of the annual rings. This is well seen in the case of the section of the Scotch fir shown in Fig. 7, Pl. XXV, for which I am indebted to Mr D. W. Steuart. The tree in question was grown at Broxburn, near the Roman Camp Shale Works, which were opened 17 years before the tree was cut down. In consequence of the acid fumes and smoke-contaminated atmosphere, the diameter growth as measured by the annual ring was sharply checked when the tree was 12 years old, though under normal circumstances it would be fairly constant up to 30 years old and then fall steadily off. This sharply defined check in the growth of the tree, as recorded by itself, is coincident with the opening of the Shale Works in 1893.

A chemical analysis of the plant will give exceedingly useful information as to the amount of smoke pollution in the district in which it was grown. In this connection the following analyses of the deposits on aucuba leaves carried out by the Air Analysis Committee of the Field Naturalists' Society of Manchester in the winter of 1890–91 are of interest.

*Deposit on Aucuba Leaves.*

In milligrams per square metre of leaf surface.

| Date | Locality | Description | Solid deposit | Sulphuric acid | Hydrochloric acid |
|---|---|---|---|---|---|
| 1890 | | | | | |
| Dec. 14 | Alexandra Park | Surburban | 131 | 7·2 | 9·1 |
| „ 13 | Owens College | „ | 315 | 10·4 | 17·3 |
| „ 16 | Hulme | „ | 420 | 26·0 | — |
| „ 14 | Harpurhey | „ | 443 | 19·0 | 4·4 |
| „ 14 | Infirmary | Urban | 728 | 27·5 | 19·4 |
| „ 13 | Albert Square | „ | 833 | 24·2 | 21·7 |
| 1891 | | | | | |
| Jan. 17 | Peel Park | Surburban | 374 | 18·0 | — |
| „ 22 | Queen's Park | „ | 194 | 17·5 | — |

It will be seen that the central localities show the largest deposits of soot and acid; the sulphuric acid there forming from 6 to 9 per cent. and the hydrochloric acid from 5 to 7 per cent. of the total deposit.

But these impurities due to smoke pollution get not only *on* the leaf but *in* it. It has already been seen that the stomata of leaves may be choked by a tarry deposit. The leaf may also absorb sulphur dioxide from the atmosphere partly through its stomata and partly in the case of some leaves possessing a thin cuticle directly through the epidermis. Hence the sulphur content of leaves will give perhaps one of the best indications of the amount of smoke pollution.

A very large number of leaves from different districts have been analysed in this way, the figures quoted are those connected with the laurel leaves referred to on p. 394, which were collected on February 15th, 1913.

| District | Annual deposit of soot in tons per sq. mile | Percentage of $SO_3$ in dry matter of leaf |
|---|---|---|
| Sutton | Free from smoke pollution | 0·31 |
| Weetwood Lane | 42 | 0·66 |
| University | 114 | 1·28 |
| Hunslet | 539 | 1·98 |

If this method is used for a comparison of the relative freedom from pollution of different districts, two or three points must be borne in

mind. The leaves must be taken from the same variety of trees or shrubs, for different types of leaves will absorb sulphur dioxide at different rates and hence possess different proportions of $SO_3$. They must also be collected at the same time for the sulphur content of leaves will increase with age. The total sulphur content expressed as $SO_3$ of sycamore leaves grown in Meanwood and analysed by Steuart was 0·48 per cent. in May and 0·75 in August.

The sulphur present in the plant will be partly protein and partly non-protein; and leaves grown in a smoke-infested area will not only contain a large amount of sulphur; but that sulphur will be present principally in non-protein forms, mainly sulphates. The following analyses of elder leaves collected in June, 1913, at the stations mentioned illustrate this point.

| Station | Yearly deposit of $SO_3$ in tons per sq. mile | Percentage of $SO_3$ in dry matter | | | Ratio | |
|---|---|---|---|---|---|---|
| | | Protein | Non-protein | Total | Protein | Non-protein |
| Sutton | about 8 | ·21 | ·07 | ·28 | 300 : | 100 |
| Weetwood Lane | 28 | ·20 | ·13 | ·33 | 154 : | 100 |
| Woodhouse Moor | 38 | ·19 | ·18 | ·37 | 105 : | 100 |
| Hunslet | 96 | ·11 | ·48 | ·59 | 23 : | 100 |

In the autumn of 1919 the Department of Agriculture of this University was asked to investigate a bad case of smoke damage to a growing crop of four acres of potatoes. My estimate of the cost of growing and harvesting the crop, drawn up from the details of cultivation supplied by the owner was £98. 18*s*. In return for this outlay of nearly £100, two tons of marketable ware were lifted and sold for £18. 18*s*.; and approximately a ton and a half of small potatoes were fed to the pigs. This would mean that the actual loss in out-of-pocket expenses incurred by the grower could not possibly be less than £70 and most probably would be nearer £80. The haulms of the potatoes all showed marked signs of smoke damage, even to the eye. A chemical examination of these showed them to possess an abnormally high sulphur content, and a very low ratio of protein to non-protein sulphur. The total percentage of $SO_3$ present in the dry matter of the leaves was as high as 0·72 per cent., of which only 0·21 per cent. represented protein sulphur, and 0·51 per cent. represented non-protein sulphur.

A chemical analysis of plants grown in smoke-infested areas will also show that they contain proportions of arsenic increasing with the amount of the smoke pollution.

A *Bacteriological* examination of soils in smoke-polluted districts will

also in many cases supply information which will be of service in determining the amount of that pollution. The acidity of the smoke will deplete the soil of its calcium carbonate and in so doing will modify to a large extent the number and activity of the soil flora. The greater the acidity of the soil the smaller the number of bacteria present in the soil, and the less their activity; the nitrifying organisms being found to be the most susceptible.

The following table gives the results of bacteriological examination of the soil taken from Garforth and exposed for three years in the districts mentioned.

| District | Annual deposit in tons of $SO_3$ per sq. mile | Calcium carbonate in soil | Total no. of bacteria per gram of dry soil | Nitrifying organisms: Ammonia converted into nitrates | Putrefactive organisms: Ammonia produced from peptone | Nitrogen fixing organisms: Nitrogen fixed per gram of mannite |
|---|---|---|---|---|---|---|
| | | % | Thousands | Mgms. | Mgms. | Mgms. |
| Weetwood Lane | 28 | 0·30 | 1536 | 8·7 | 105 | 26 |
| Headingley | 33 | 0·26 | 1236 | 6·4 | 88 | 21 |
| University | 38 | 0·19 | 1054 | 4·3 | 78 | 19 |
| Park Square | 56 | 0·17 | 876 | 1·9 | 67 | 18 |
| Hunslet | 96 | 0·12 | 798 | 1·2 | 64 | 15 |

These results indicate clearly that the detrimental effect of the smoky atmosphere upon plant growth is partly due to unfavourable changes in the soil—such as the steady depletion of the stock of calcium carbonate, and the inhibition of the activities of the nitrogen-adapting soil flora.

We may notice that plants grown in a smoke-polluted atmosphere lose their vitality; but it is possible in a large number of ways to get a measure of that loss of vitality and to use the information thus obtained to gauge the amount of smoke pollution in different districts.

This loss of vitality will be shown in a diminution of the *Reproductive Powers of the Plant*, whether propagated from seed or from cuttings.

(*a*) The following table gives the germination capacity of oats grown in 1913 at the stations indicated.

| Station | Annual deposit in tons per sq. mile | Germination capacity (10 days) |
|---|---|---|
| Adel | 29 | 98 % |
| Headingley | 78 | 92 |
| University | 114 | 64 |
| Hunslet | 539 | 17 |

(*b*) 100 viola cuttings taken from plants grown in Roundhay (annual deposit 26 tons per square mile) were struck in Hunslet, and 98 per cent. grew.

100 viola cuttings taken from plants *grown* in Hunslet were struck in Hunslet, but not a single cutting grew.

It is shown in the *smallness* and *lack of weight of seeds* of plants grown in a smoke-infested area.

*Weight of* 100 *corns of barley grown from same seed.*

| Where grown | Annual deposit in tons per sq. mile | Weight of 100 corns |
|---|---|---|
| Easingwold | Free from smoke pollution | 4·85 gms. |
| Adel | 29 | 4·81 |
| Headingley | 78 | 4·35 |
| University | 114 | 2·27 |
| Hunslet | 539 | 1·16 |

It is shown not only in the diminution of the germination capacity, but also in the diminution of the *Germination Energy*.

It is shown in the inability of the plant to put up a fight against adverse conditions, as for example, the winter frosts. To test this point nine cabbage plants were planted out in the autumn of 1913 at the five stations mentioned.

| Station | Annual deposit in tons per sq. mile | Observation |
|---|---|---|
| Weetwood Lane | 42 | 8 out of 9 survived the winter |
| Headingley | 78 | 2 out of 9 survived the winter |
| University | 114 | 6 dead by Christmas. Frost in February killed rest |
| Park Square | 243 | All dead by middle of November |
| Hunslet | 539 | All dead before end of October |

Where the total annual smoke deposit exceeds 100 tons per square mile, it is dangerous to adopt autumn planting either of wallflowers, cabbages or spinach. Where that deposit exceeds 200 tons per square mile it is absolutely fatal. More than 100 wallflowers were planted out in Hunslet in September and not one stood the winter. They can be grown in smoke-polluted districts but they must be planted out in the spring.

The loss of vitality is shown perhaps most noticeably in the inhibition of the activity of the various *enzymes* or ferments which assist in the chemical processes taking place in the plant. The figures given in the following table refer to laurel leaves collected in February, 1913; and illustrate the way in which the activity of the various enzymes are influenced by smoke pollution.

| District | Annual deposit in tons per sq. mile | Oxidase activity | Catalase activity | Emulsin activity |
|---|---|---|---|---|
| Sutton | Free from smoke pollution | 100 | 100 | 100 |
| Weetwood Lane | 42 | 64 | 47 | 85 |
| University | 114 | 24 | 37 | 51 |
| Hunslet | 539 | 0 | 34 | 35 |

The relative lipase activity in the oat grain grown in 1915 in the districts mentioned was measured by allowing the enzymes present in the grain to hydrolise an ethereal salt like ethyl butyrate or ethyl acetate and observing the amounts of acid liberated by titration against $N/10$ NaOH. The results show that the activity of this enzyme as well as that of the others previously mentioned is inhibited by smoke pollution.

*Effect of Smoke Pollution on Activity of Lipase in Oat Grain.*

| District | Relative activity of lipase |
|---|---|
| Adel | 100 |
| Weetwood Lane | 75 |
| Headingley | 66 |
| University | 54 |
| Hunslet | 42 |

In conclusion, I should like to express my indebtedness to Professor Crowther and Professor Cohen for invaluable help and guidance all through the investigations; to Mr Hector for assistance given in the microscopic examination of many of the leaves; to Mr Frank for help given in the measurements of the tints of the flowers; to Professor Priestley for guidance in the estimation of enzyme activities; and to all those gentlemen who have so kindly placed their gardens at my disposal for so many years.

## EXPLANATION OF PLATES XXIV, XXV

Fig. 1. Oats from same sample of seed grown in different districts in and round Leeds, Adel, three miles to north of Leeds, Hunslet, Industrial Leeds.

Fig. 2. Sycamore.

Fig. 3. Lime.

Fig. 4. Ribes. Smoke-damaged leaves collected 29 May, 1914; from Woodhouse Moor, one mile to the north of Leeds.

Fig. 5. Holly leaf obtained from tree growing in industrial Leeds. (Upper half cleaned: lower half showing deposit of soot.)

Fig. 6. Leaves of Conifers, collected at Garforth, showing "sunk stomata" choked with tar.

Fig. 7. Section of Scotch fir, grown at Broxburn, showing damage done by fumes from Roman Camp Shale Works.

Fig. 1.

Fig. 2.

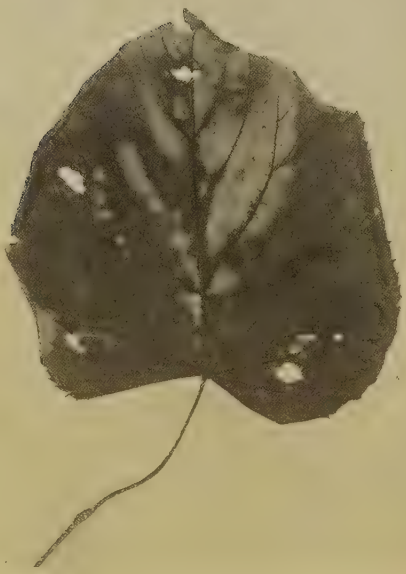
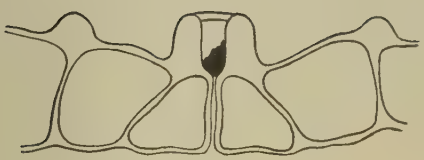


Fig. 3.

Fig. 4.



Stoma of juniper.

Stoma of silver fir.

Fig. 6.

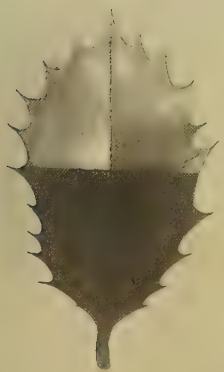


Fig. 5.

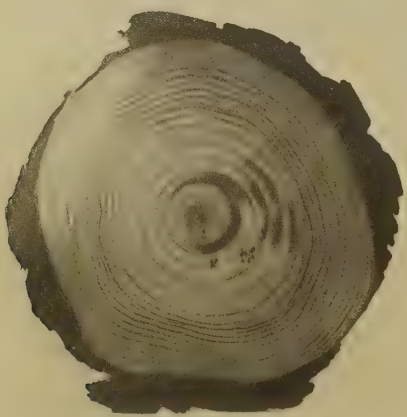


Fig. 7.

# METHODS IN THE QUANTITATIVE ANALYSIS OF PLANT GROWTH—A REPLY TO CRITICISM.

By G. E. BRIGGS, F. KIDD and C. WEST.

The Chief Statistician at the Rothamsted Experiment Station in his criticism of the methods put forward by us in recent articles(4 & 5) on The Quantitative Analysis of Plant Growth, summarises his remarks as follows. "The methods of calculation formulated by Briggs, Kidd and West for the analysis of plant growth are inaccurate." We feel that this sweeping statement standing in the forefront of the conclusions of what might appear to be an authoritative utterance by a statistician cannot be allowed to pass unanswered, the more so in that it is not supported by evidence or argument in the body of Mr Fisher's paper.

## *The Method.*

The general method proposed by us for a quantitative analysis of plant growth is to obtain from primary data recorded at frequent intervals throughout the life of the plant, secondary relations[1]. We proposed the following as being probably the most useful at the outset.

1. Relative Growth Rate.
2. Leaf-Area Ratio.
3. Unit Leaf Rate.
4. Relative Leaf Growth Rate.

We suggested that by a careful comparison of these with each other and with records of environmental factors one might be able to disentangle problems of plant growth and thus eventually to evaluate plant constants. We do not wish it to be concluded that we proposed these as the only significant secondary relations. For example, from an analysis of the results of growth experiments carried out by us more recently it appears that the ratio of the rate of growth to the rate of respiration is a further significant secondary relation.

The question of the value of this general method is not dealt with by Mr Fisher, and indeed can only be decided by the results obtained from its application.

[1] For details of these secondary relations the reader is referred to (5).

*The Methods of calculating Secondary Data.*

The question then arises, are the methods put forward by us for calculating the above secondary relations inaccurate? This seems to be the second possible interpretation of Mr Fisher's summary and is, moreover, what he literally states. In the first place, in spite of the conclusion quoted above, Mr Fisher deals only with the methods proposed for calculating Relative Growth Rate, $R$, and says nothing about the methods for calculating the other secondary data mentioned above. We defined $R$ ((5), p. 204) as the weekly percentage rate at which the dry weight increases, and stated that if the rate were continuous compound interest the equation for calculating $R$ would be

$$\frac{R}{100} = \log_e W_2 - \log_e W_1 \quad \text{.....(1),}$$

and if the rate were simple interest the equation would be

$$\frac{R}{100} = \frac{W_2 - W_1}{W_1} \quad \text{.....(2).}$$

Both these methods of calculation were put forward(5)[1]. Mr Fisher urges the use of the first, and states the second to be inaccurate. As a matter of fact both methods of calculation are perfectly in concordance with our definition and neither of them can be in itself characterised as inaccurate.

*The Use of Relative Growth Rate Values.*

A third possible explanation of Mr Fisher's criticism is that, instead of meaning the charge of inaccuracy to apply to our methods of calculation, he means it to apply to the use we have made of the values of the Relative Growth Rate calculated by means of equation (2). So far we have published a paper(4) in which the fact has been recorded that the Relative Growth Rate, calculated by *either* of the two methods described above, follows the generalised form of curve shown in Fig. 9 of the paper in question, and that the Leaf-Area Ratio curve follows a closely similar course. It was pointed out at the time that, for the purpose of demonstrating this fact, it is immaterial which of the two methods of calculation is utilised, and indeed, in Fig. 1 a comparison was made of the results obtained by the two methods. Mr Fisher has only

[1] The definition of Relative Growth Rate given in (4, p. 105) is for the rate calculated on the simple interest basis. It must be understood that we do not suggest that the growth of a plant is a process of accumulation of dry-weight at either "continuous compound" or "simple" interest. On the one hand, the whole of the new material is not put out as new capital, and on the other hand there is an unknown time interval before any new material can become new capital.

elaborated this comparison. It was decided to present the majority of the results in that paper in the form adopted (*i.e.* calculated by equation (2)) in order that the calculation might be perfectly intelligible to non-mathematical readers. Mr Fisher's attack centres round this point and amounts to a statement that for statistical purposes the results obtained by equation (1) are preferable to those obtained by equation 2. Since we did not attempt any statistical correlations in the paper under consideration his criticism is irrelevant.

Miss Brenchley, with Mr Fisher's help, has utilised Relative Growth Rates calculated by equation 1 for determining the correlation between growth rate and temperature and sunshine respectively (3). We agree that the values calculated from our first formula (equation 1) are the values to be utilised for statistical correlations provided due consideration be given to the complexity of the problem. We propose to consider this question in detail in another place.

The explanation of Mr Fisher's misunderstanding is most probably to be traced to the discrepancy between our definition of Relative Growth Rate and Mr Fisher's and to the fact that he imputes his definition to us. While we define the Relative Growth Rate as the weekly percentage rate at which the dry-weight increases, in a previous number of this *Journal* ((4), p. 104) we pointed out, as Mr Fisher himself quotes, that "the principle of the proposed method of expressing rate of growth is analogous to that of the method by which the rate of most reactions, both chemical and physiological, are expressed, namely, amount of change per unit of material per unit of time." This precise physico-chemical definition, to which we say our definition is only analogous, Mr Fisher adopts as a definition of Relative Growth Rate and imagines we have done likewise, which we have not. Our attitude is that until we gain a more thorough knowledge of the complexity of the processes involved in plant growth, the adoption of a definite physico-chemical conception is not warranted since it may lead to the mistaken impression that its adoption constitutes in itself an advance in physiological knowledge.

His charges of "inconsequent arbitrariness in method of calculation when contrasted with precision of definition," and his imputation that our choice of method of calculation is explainable by "the mistaken impression that the use of the logarithmic formula involves the assumption that the relative rate of increase is independent of time," when viewed in the light of the above discrepancy of definition can be readily explained.

With regard to the second paragraph of Mr Fisher's summary we do not wish to reopen the discussion on the significance of the "Efficiency Index." Equation (1) given above for calculating relative growth rates is the same as Blackman's formula for calculating the "Efficiency Index(1)." There is nothing original in the formula itself, but whereas we propose an analysis by an evaluation of the Relative Growth Rate for weekly or for shorter periods[1] throughout the life-cycle, Blackman(1) and Brenchley(2) used this formula indiscriminately for periods of widely varying length and covering widely different portions of the life-cycle.

Botany School,
Cambridge.

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[1] We have used weekly periods as no data for shorter periods are at present available. Daily or half-daily measurements would provide data for a much deeper analysis.

# A TOMATO CANKER.

By ETHEL M. DOIDGE,

*(Assistant Chief and Senior Research Officer, Division of Botany, Pretoria.)*

(With 5 Text-figures and Plate XXVI.)

## Introduction.

A disease which may be described as a canker of tomatoes was first noticed in fruit on the Pretoria market during the summer of 1914. It is most prevalent from January to March, and during that period each year (1914–20) a large percentage of the fruit offered for sale was disfigured. Affected fruit rapidly becomes attacked by soft rot organisms which enter the fruit through cracks caused by the scab lesions and destroy it within a few days.

The incidence of the disease has a direct relation to the rainfall. Tomatoes which ripen in the early summer are seldom attacked, being grown under irrigation, and the disease is seldom observed up to the end of November. From November to January the temperature is high, and there is usually a considerable amount of rain; moist, humid conditions are favourable to the development of the disease. The fruit can only become seriously scabbed when it is attacked by the organism in the early stages of its development; this would account for the non-appearance of the disease before December or January. A few lesions are to be found on fruits as late as June, but the disease can be regarded as serious only during the summer months.

The occurrence of canker in fruit on the Pretoria market was suggestive of the fact that the disease occurred in market gardens in the district; several tomato growers were visited and the surmise found to be correct. Canker is a very common trouble in market and private gardens in the Pretoria district; it is reported to occur also in the Rustenburg district, but I have seen no specimens from there, and beyond this nothing is known of the distribution of the disease. It is not regarded as being of a very serious nature, but a large percentage of the fruit is disfigured, and much of it decays if it is kept for a few

days after ripening. The summer of 1919–20, when most of the observations were made, was exceptionally dry: another season with heavier or more continuous rainfall the losses would probably be far more serious, and might ruin the whole crop.

The "canker" lesions differ from those caused by other tomato diseases attributed to bacteria. The wilt disease caused by *Bacterium solanacearum* Erw. Sm.(3) is a distinctly vascular trouble and causes a characteristic wilting of the plants. *Aplanobacter michiganense* is also found in the vessels(2). "Streak," described by Paine and Bewley as caused by *Bacillus Lathyri*, is characterised as the name suggests "by the formation of dark brown or black sunken patches on the stem," varying from small spots to long furrows or blazes(1)." On the fruit it forms light or dark brown sunken patches with round or irregular outline.

The effects of the canker organism on leaf, stem and fruit are widely different from any of these as will be evident from the detailed descriptions to be given later.

## Symptoms of Disease.

On the leaves the first indication of infection is the appearance of numerous dark green, semi-translucent, water-soaked points on the under surface. In cases of artificial infection in autumn weather this occurred seven to eight days after inoculation, under summer conditions the progress of the disease may be more rapid. The spots increase in size and become round or irregular and about 2 mm. in diameter; they are slightly sunken and are often present in such numbers in the neighbourhood of the lateral veins and leaf margins, that they coalesce, and produce irregular, discoloured streaks. The colour soon changes from dark green to deep quaker drab[1] (Ridgway 51) or vinaceous slate (50). The discolouration penetrates to the upper surface, and the spots eventually consist of a smoke grey centre, which is then membranous and semi-translucent surrounded by a deep brownish drab margin.

Where the spots are numerous the intervening leaf tissue becomes dry, brown and brittle, the original lesions being still plainly visible in the dead areas. In this way the affected portions of the leaf, especially the edges and the tips, become dead and dry and break away, giving the leaves a very ragged appearance, and many of the smaller leaflets are

[1] The numbers quoted after name of colours refer to plates in Ridgway's *Colour Standards and Nomenclature.*

altogether destroyed. Affected leaves show a tendency to curl inwards, and are more or less twisted and distorted.

Spots on calyces, pedicels and young parts of the stem are similar in character to the leaf spots. On the calyx they may be numerous, but very minute and scattered, or less numerous and up to 2–3 mm. diameter, forming elongated streaks up to 5 or 6 mm. long.

Cankers are produced on older parts of the stem, especially where the tissues have been somewhat injured by friction or otherwise. At first there appear irregular, dark green water-soaked areas, which later become corky-looking, slightly raised, roughened and with numerous small longitudinal cracks. They are nearest tawny olive in colour. The surface has the appearance of having become blistered or raised by abnormal tissue development underneath, with subsequent cracking of the blistered areas. Cankers of irregular form and 1–2 cm. in diameter are not uncommon. The discoloration does not penetrate into the wood; it is apparently confined to the cortex and is quite superficial.

In the field, infected fruits are usually found immediately below diseased leaves and are doubtless infected during rainy weather by raindrops which fall on infected leaves and subsequently drip on to the young fruit. The majority of the fruit spots are at the stalk end, but they are also found scattered over the sides and, less frequently, on the blossom end.

A very minute green or brownish blister is the first indication of infection: this blister may remain minute, about 1 mm. diameter, or may increase in size up to about 3 mm. and become considerably raised above the normal fruit tissue. Occasionally, presumably when weather conditions are unfavourable, infection does not proceed further, and when the fruit is ripe these minute blister-like spots have almost the appearance of fly-specks (Plate XXVI, fig. 2).

In the large majority of cases the point of infection becomes surrounded by a dark green, water-soaked area which spreads considerably and then begins to discolour from the centre. The centre becomes deep slaty brown, merging into wood brown at the edges; a water-soaked margin about 1 mm. wide is still apparent whilst the organism is active. Finally the epidermis ruptures in the centre, showing whitish brown over the discoloured tissues like the broken edges of a blister. The spots are hard and scabby in texture and usually slightly convex, although in mature fruit they may lie in slight depressions owing to arrested growth at the point of infection (Plate XXVI, fig. 1).

Single scabs are usually not more than 5 mm. diameter, but they

are often so numerous and close together that they coalesce, forming large, scabby areas several centimetres in extent. As the fruit ripens the tissues round the infected areas remains green, forming a green rim round the scabs which is conspicuous on the red fruit. The rifts in the epidermis become extended in cases of severe infection and whitish brown cracks are formed, many of them over 1 cm. in length and extending into unaffected tissue. These open the way for putrefactive organisms and the fruit usually rots within a few days after ripening. Thus the disease not only disfigures the fruit and reduces its market value, but seriously affects its keeping qualities.

Fig. 1. Early stages in leaf infection.

### Morbid Anatomy.

In inoculation experiments tomato leaves and fruit readily became infected without wounding, so that in all probability infection usually takes place through the stomata. This was confirmed by an examination of a large number of sections of spotted leaves: in the earliest stages of infection the bacteria are to be found in the sub-stomatal cavity (Fig. 1 A), and later make their way to the adjoining intercellular spaces. The middle lamella of the cells becomes much swollen, and stains an intense red in sections treated with carbol fuchsin and light green, the cells in the surrounding healthy tissue staining green (Fig. 1 B). As described by

Paine in connection with the "streak disease(1)," the cells become torn asunder by shrinkage of dead cells and by the tension set up by growth of the surrounding healthy tissue and eventually large cavities are produced. There are not many living bacteria to be found in the disorganised tissues, but at the edges of such lesions, where the organism

Fig. 2. Section through small lesion on fruit.

is still active, large pockets of bacteria may be found. In the leaf the organism penetrates through the thickness of the leaf, entirely disorganising the tissue, but it does not travel far in a lateral direction. Stem lesions are very similar, but the organism has not been found to penetrate beyond the cortex.

In the fruit a similar disorganisation of cells takes place, but the hypodermal cells at the edge of the injured tissue begin to divide actively (Fig. 2) and sometimes form a complete layer of new cells under the disorganised tissue (Fig. 3); this accounts for the convex contour of the canker. Sorauer (5) has illustrated a similar condition as a result of hail injury; it is probable therefore that the multiplication of cells is not due directly to the action of the organism but to the presence of dead tissue in the rapidly growing fruit.

Fig. 3. Section through lesion on fruit, showing complete layer of actively dividing cells.

The nuclei in the affected cells become much hypertrophied, becoming five or six times the size of nuclei in healthy cells. The nucleolus becomes very large and vacuolate, and the nucleolar membrane disappears. There are frequently two nuclei in a cell or two nucleoli in one nucleus, the latter frequently irregular or lobed, being similar in shape to nuclei dividing amitotically as found by Smith (4) in crown gall lesions; but there was no clear evidence that the nuclei were dividing in this way.

In the entirely disorganised cells the nucleus continues to increase in size until the nuclear membrane is ruptured and the chromatin becomes diffused; the nucleolus remains intact for a considerable time, taking a red stain and standing out clearly in the disorganised mass.

## ISOLATION OF THE CAUSAL ORGANISM.

The organism was first isolated in January, 1914, from tomatoes purchased on the Pretoria market. On these plates a yellow organism was predominant, but there were also colonies of a spreading, cream-coloured organism. At that time no greenhouse accommodation was available, and attempts to inoculate tomato plants out of doors during a spell of dry weather were unsuccessful; it was quite impossible to keep the atmosphere sufficiently moist. Attempts to inoculate detached fruits in the laboratory also proved a failure.

The disease was noticed on fruit exposed for sale each summer, but there was no further opportunity to investigate the matter until February, 1920. The organism was again plated out from fruit exposed for sale on the Pretoria market; colonies were visible after 48 hours, at 30° C., and in three days assumed their characteristic form and yellow colour. An almost pure culture was obtained direct from the host. The colonies were very similar in appearance to those of *Bacterium citri*, *B. campestre* and other related organisms; there appears to be a large number of plant parasites belonging to this group.

During March, 1920, the organism was repeatedly isolated from scabby fruit and infected leaves collected in a market garden at Daspoort, near Pretoria. In each case a pure culture of the organism was obtained without difficulty, from both leaf and fruit. It is more easily isolated from the fruit since the surface can be sterilised and the culture made from the tissue underneath.

## INOCULATION EXPERIMENTS.

### *Preliminary Experiments.*

#### A.

*28. 1. 20.* Two well-grown tomato plants were used bearing green fruit, almost full grown. The fruits were inoculated with a culture isolated from tomato fruits purchased on the Pretoria market. The culture was applied with a camel's hair brush, a few tomatoes on each plant being pricked and the rest uninjured. Inoculated fruit was covered for 24 hours with beakers covered with brown paper and plugged with cotton wool.

*12. 2. 20.* Dark green, water-soaked areas round needle pricks.

*16. 2. 20.* Two tomatoes ripened and were removed from plant; tissue round needle prick has remained green with a slight tendency to brown discolouration and are crowded with bacteria.

*25. 2. 20.* Remaining fruit ripened and picked. All calyces show numerous infections, but fruit infections have not gone beyond the green, water-soaked stage. Stems show extensive cankers where they were slightly injured by friction of beaker placed over fruits after inoculation. Control plants remained sound and showed no sign of infection.

B.

*10. 3. 20.* Three large tomato plants were used, bearing fruit at various stages of development. Some fruits were left uninjured, others pricked or lightly scratched. The culture was applied to fruits and leafy tips with a camel's hair brush, and the plants atomised with water. The leaves were left uninjured. All inoculated portions were covered for 24 hours with butter paper bags, to prevent excessive drying.

*13. 3. 20.* Numerous minute light and dark brown specks on younger fruits.

*18. 3. 20.* Numerous minute, water-soaked spots on leaves, also indications of infection round needle pricks on fruit.

*23. 3. 20.* Tomatoes which were very young when inoculated and which were not pricked show minute brownish blisters all over the surface. One which had only just set when inoculated had an irregular water-soaked zone round the blisters up to 2 mm. diameter. Leaf infections increasing in size and number and assuming characteristic purplish grey colour. Infected spots also visible on calyces, pedicels and stems.

*29. 3. 20.* Spots on small tomato mentioned above beginning to discolour: water-soaked zone round blisters on slightly larger fruit and round needle pricks on fruit which was inoculated by puncture when almost full grown.

*12. 4. 20.* Scabs on all fruits now quite typical; they have discoloured, become brown, epidermis has blistered and cracked in several directions. Badly infected leaflets become entirely yellow and dead.

*13. 4. 20.* Plants discarded. Control plants showed no sign of infection.

C.

*29. 3. 20.* Eight young plants about 1 ft. high were used. They were inoculated by atomising with culture isolated from artificially infected plant described in experiment B.

*6. 4. 20.* Numerous minute, water-soaked areas observed on younger leaves.

*13. 4. 20.* Leaf spots have assumed typical form and colour. Control plants showed no sign of infection.

D.

*10. 5. 20.* One tomato plant in bearing was inoculated. Fruits of various sizes and leafy tips were inoculated without wounding, and covered with butter paper bags for 24 hours.

*19. 5. 20.* Small raised spots visible on fruits, water-soaked spots on leaves. (The majority of the affected fruits and leaves were removed for purpose of studying morbid anatomy.)

*Cross Inoculations.*

It has been mentioned that the organism belongs to what Smith once termed the "yellow *Pseudomonas* group." Since five other organisms of this group were under observation at the time, and all are very similar in culture, it was thought advisable to carry out a series of cross inoculations. All these organisms infect their hosts by way of stomata or water pores; the inoculations were therefore made without wounding the plants.

A.

*24. 3. 20.* Six tins (1–6), each containing four young tomato plants, were used. These plants were about 1 ft. high and growing vigorously. One set of four plants (1) was inoculated with a culture of *Bacterium campestre*, one (2) with *B. citri*, and one each with (3) *B. Juglandis*, (4) *B. Phaseoli*, (5) *B. malvacearum*, and (6) the tomato organism.

*31. 3. 20.* Numerous minute, water-soaked spots on tomato seedlings inoculated with organism (6) from tomato scab. Other plants show no infection.

*6. 4. 20.* Spots on tomato seedlings in tin (6) have now assumed characteristic form and colour. All plants in tins (1–5) perfectly sound and showing no signs of infection.

*13. 4. 20.* No further development.

B.

*24. 3. 20.* Six tins of lemon seedlings were used, each containing six plants 10–15 inches high. One group of six seedlings was inoculated with each of the organisms mentioned in experiment A. The tins were numbered (1–6) as follows: (1) inoculated with *Bacterium campestre*, (2) with *B. citri*, (3) *B. Juglandis*, (4) *B. Phaseoli*, (5) *B. malvacearum*, (6) *Bacterium* causing tomato scab.

*30. 3. 20.* Incipient cankers noticed on lemon seedlings (2) inoculated with *B. citri*.

*7. 4. 20.* Cankers on seedlings (2) increasing in size and number. No sign of infection on plants inoculated with other organisms.

*15. 4. 20.* Numerous typical citrus canker lesions on leaves and stems in tin (2). All other plants have remained sound and show no sign of infection.

C.

*24. 3. 20.* Six cotton plants 2 ft. to 2 ft. 6 in. high were employed, one plant being inoculated with each of the six organisms mentioned in connection with experiments A and B.

*29. 3. 20.* Numerous minute, water-soaked spots on young leaves of plant (5) inoculated with *B. malvacearum*.

*7. 4. 20.* Spots have increased in size and assumed characteristic angular outline.

*15. 4. 20.* Leaf spots on plant (5) have discoloured, and are now dark brown or black. Cotton plants (1–4) and (6) inoculated with other organisms show no sign of infection.

D.

*14. 4. 20.* Six young walnut trees planted in separate tins were numbered (1–6) and inoculated with the six organisms tested in experiments A–C.

*19. 4. 20.* Numerous minute water-soaked spots on young leaves on tree (3) which was inoculated with *B. Juglandis.*

*5. 5. 20.* Spots on tree (3) have discoloured and assumed typical appearance of walnut blight lesions. All other trees remained sound and showed no sign of infection.

E.

*14. 4. 20.* Six tins each containing four well-grown cabbage seedlings were used; the tins were numbered (1–6), the four plants in each being inoculated with one of the same six organisms.

*24. 4. 20.* There is an indication of blackening of veins at edges of some of the leaves in tin (1), plants inoculated with *B. campestre.*

*12. 5. 20.* Cabbage plants in tin (1) show typical black rot lesions. No sign of infection in any of the other tins (2–6).

F.

*14. 4. 20.* Four bean seedlings were inoculated with each of the six organisms. Tins again numbered (1–6), four seedlings in each, and inoculated in the same sequence as in experiment A.

*19. 4. 20.* A few water-soaked areas visible on leaves of plants in tin (4) inoculated with *B. Phaseoli.*

*20. 4. 20.* Very numerous points of infection now visible on plants in tin (4).

*30. 4. 20.* Leaves of bean plants in tin (4) now heavily infected and showing numerous typical bean blight lesions. No sign of infection on plants in tins (1–3) and (5–6) inoculated with other organisms.

*Inoculation on to other Hosts.*

A.

*7. 4. 20.* 12 tobacco seedlings about six inches high were atomised with a culture of the tomato organism.

*13. 4. 20.* No sign of infection.

*7. 5. 20.* Still no sign of infection—plants discarded.

B.

*7. 4. 20.* Two plants of *Physalis minima* were inoculated by atomising with a suspension of a potato culture.

*13. 4. 20.* Numerous minute water-soaked spots observed on younger leaves.

*17. 4. 20.* Spots have increased in size, are somewhat angular, and are becoming brown. Control plants show no sign of infection. Re-isolated organism from infected leaves and found it to be identical with the original.

C.

*7. 4. 20.* One plant of *Datura stramonium* var. *tatula* was inoculated by atomising with a suspension of a culture of the tomato organism.

*13. 4. 20.* Numerous minute, water-soaked points on younger leaves: tissues on examination prove to be full of bacteria.

*17. 4. 20.* On *Datura* plant inoculated, organism has produced very numerous spots about 1 mm. diameter and somewhat angular, these are brown and membranous in centre and have a water-soaked margin: these brown spots show a tendency, especially in the younger leaves, to break away and cause a shot-hole effect. Control plant is healthy and shows a sign of infection; replated organism from infected leaves and found it identical with original.

D.

*7. 4. 20.* One plant of *Solanum incanum* inoculated without wounding.

*17. 4. 20.* No signs of infection on this or later date; leaf is protected by thick covering of hairs.

E.

*7. 4. 20.* One plant of *Solanum nigrum*, inoculated without wounding.

*13. 4. 20.* Slight indications of infection on younger leaves.

*17. 4. 20.* Infected spots have developed considerably, but are still small: they are irregular in outline, 1 mm. diameter or up to 2 mm. long, and are light brown and membranous in the centre. Replated organism and found it identical with original. Control plant showed no sign of infection.

F.

*24. 7. 20.* A number of sweet pea plants were inoculated by needle pricks in the stem. The result was entirely negative.

## DESCRIPTION OF THE CAUSAL ORGANISM.

### A. *Morphological Characters.*

When taken direct from the tissues of the host the organism is very variable in size and form: the majority are rather stout rods with rounded ends, but some are so short as to resemble coccus forms. They vary from ·6 to 4 $\mu$ in length, and from ·5 to ·75 $\mu$ in breadth. The majority are 1–1·5 × ·6–·7 $\mu$.

In young agar cultures (24 hours at 30° C.) the organism is a rod with rounded ends, occurring singly or in pairs, or less frequently in short chains of three to seven elements. The rods are for the most part fairly even in size, measuring 1–1·5 × ·6–·7 $\mu$; the extremes observed were ·8–3 × ·6–·7 $\mu$. In old cultures they are less regular, and short forms are numerous.

In young bouillon cultures the rods are similar in form. The organism grows rather reluctantly in neutral bouillon, and in six days old cultures in this medium there are numerous straight or curved rods 4 and 5 $\mu$ long and also long undivided threads up to 50 $\mu$ long.

On gelatine the average length is slightly greater, being 2–3 $\mu$, and the breadth is ·6 to ·65 $\mu$. Limits observed were 1–4 × ·6–·7 $\mu$. The rods were mostly single but occasionally in pairs or short chains.

In 24 hours old potato cultures the rods are also rather long as compared with those on agar cultures; the majority are 1·5–2·5 × ·6 $\mu$. There are numerous pairs and short chains, and occasionally long threads up to 40 $\mu$ long which are only partially or imperfectly segmented. After six days the size and grouping remain the same, with the exception of the long threads which are no longer seen.

*Motility.* The organism is actively motile in very young cultures. In hanging drop cultures it moves with a forward screwlike action, its progress being frequently interrupted by rotations on its short axis

Fig. 4. Rods from streak incubated for 18 hours at 20° C. Stained by Löffler's method.

and other tumbling movements. The rods gradually made their way to the edge of the drop and came to rest. The organism is not motile as it diffuses from the tissues of the host. Rods from a young agar culture examined with the dark ground illumination showed no highly refractive granules, and the rods are apparently motile by means of a polar flagellum on the forward end which whirls like the propellor of an aeroplane.

This observation was confirmed by the examination of stained preparations. Good preparations were not very easy to obtain as the organism sheds its flagella very readily; fairly good results were obtained with a modification of Löffler's method and by van Ermengen's method. There is a single polar flagellum with a length of about 5–10 $\mu$ (Fig. 4).

*Spores* were not observed.

*Capsules.* The viscid nature of the growth on agar and other media points to the presence of a capsule. This is most clearly seen in potato cultures. Examined after 24 hours at 30° C. each rod is surrounded by a white halo; when stained with carbol fuchsin the rod stains intensely and the capsule is pale with a feebly stained margin (Fig. 5). As the culture grows older the capsule becomes more evident, and when stained with carbol fuchsin the rod and capsule stained as described above are embedded in a slimy mass which stains pink.

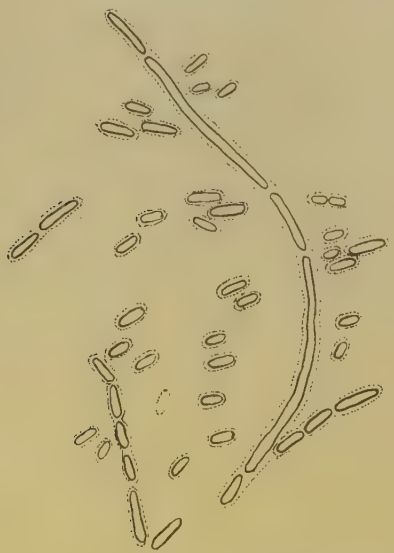

Fig. 5. Rods from potato culture, 24 hours at 30° C. Stained with carbol fuchsin.

*Involution forms* have been found in old gelatine cultures. They vary much in form; some are stout rods about 1 $\mu$ thick in the centre and tapering to pointed ends: others are longer threads up to 35 $\mu$ long and varying in thickness, curved and distorted and with or without constrictions at irregular intervals.

*Staining reactions.* The organism as taken from young cultures stains evenly and intensely with carbol fuchsin and all the ordinary aniline stains. In rods from older cultures all staining is fainter and more irregular. It is not acid fast and stains only faintly with Löffler's blue; it is Gram positive: no granules were observed in rods stained by Neisser's method.

*Cultural Characters.*

Preliminary cultivation was carried out as suggested by the Society of American Bacteriologists. In all cases, unless otherwise stated, cultures were incubated at 30° C.

*Plate cultures on nutrient agar* +15. Colonies show as minute, shining points after 48 hours. By the third day they have increased somewhat in size, and are coppery, translucent by transmitted light, and yellowish or somewhat opalescent by reflected light. There is seldom any development after the fourth day; on thinly sown plates fully developed surface colonies are circular or oval, up to 5 mm. diameter, semi-translucent, colour Naples yellow (XVI). The margin is entire and texture homogeneous, finely granular as seen under the low power of the microscope. In some there is a faint indication of concentric zoning, but this is more evident in the bluish white colonies about 2 mm. in diameter, formed between the agar and the bottom of the plate. Long feathery crystals begin to form in the medium after about ten days. Submerged colonies are minute and lenticular, and often form surface colonies by excentric development.

*Streak cultures on nutrient agar* +15. After 24 hours the streak is smooth, slightly convex, wet-shining, with entire margin, and about 5 mm. broad. The growth increases somewhat up to the third or fourth day, entirely covering the wetter part of the medium at the base of the tube. The colour is Naples yellow, and growth shows irregular, semi-opaque streaks when held up to the light. There is a heavy growth in the condensation water. Crystals begin to form under the streak at the end of the first week.

*Stab cultures in nutrient agar* +15. Surface growth only; no growth in the depths of the medium.

*Shake cultures in nutrient agar* +15. Numerous surface colonies develop and become confluent, entirely covering surface. There are some very minute submerged colonies just below the surface but none in the depths of the medium.

*Plate cultures on nutrient gelatine* +15. All gelatine cultures were incubated at 20–22° C. Colonies were visible on the third day; on the fourth day thickly sown plates were partly liquefied, on more thinly sown plates surface colonies were up to 1 mm. diameter, convex glistening, cream colour, translucent, context homogeneous and finely granular; submerged colonies minute, opaque, lenticular. On the sixth day thickly sown plates were completely liquefied; in more thinly sown plates the

colonies were up to 5 mm. diameter and each sunken in a saucer of liquefaction, the liquefied gelatine was clear and the colonies denser in the centre than at the circumference. On the tenth day the gelatine in the thinly sown plates was completely liquefied, the medium being very slightly clouded. Colonies were still intact but somewhat diffused, up to 15 mm. diameter and often rolled at the edges; they were not tough, but gelatinous in consistency, breaking very readily if an attempt were made to lift them with a needle. Under the low power of the microscope the centre was moruloid to grumose.

*Stab cultures in nutrient gelatine* +15. After 24 hours there were slight indications of growth at the surface and along the upper part of the stab, and on the third day there was a shallow crater of liquefaction at the surface. On the fourth day liquefaction was crateriform, 7–10 mm. wide and about 5 mm. deep; there was a good surface growth and a slight bacterial deposit on the unliquefied gelatine, but the liquefied part of the medium was colourless. Subsequently liquefaction became stratiform, and when it had proceeded to a depth of about 1½ cm. growth apparently ceased. The gelatine remained clear or very slightly cloudy, and eventually the surface growth sank to the bottom of the liquefied portion.

*Streak cultures on nutrient gelatine* +15. A whitish streak about 1 mm. wide is visible after 24 hours, which is replaced on the second day by a narrow channel of liquefaction along the needle track; this channel gradually becomes more extensive, the bacterial growth being carried with the liquefied gelatine to the bottom of the tube. This continues until there is 1–1·5 cm. of liquefied gelatine at the bottom of the tube.

*Shake cultures in nutrient gelatine* +15. Numerous minute surface colonies appeared in the third day, and the gelatine was slowly liquefied from the surface downwards, liquefaction being first noticed on the fourth day. At no time was there any growth in the depth of the medium.

*Potato.* On steamed potato cylinders a spreading, wet-shining butyrous growth covered the moister parts of the cylinder in 24 to 48 hours; on the sloping surface there was a broad, more or less raised streak surrounded by a narrow white "fermentation zone." The growth was amber yellow, and became somewhat viscid, especially in old cultures; it increased in quantity, covering the whole cylinder unless the medium was dry; the colour deepened somewhat with age and the medium was somewhat greyed.

*Carrot.* On steamed carrot the organism produces in three days a spreading, wet-shining, cream-coloured growth, practically covering the medium, and a thick shiny growth on the surface of the liquid at the bottom of the tube; the colour deepened after the first few days, becoming straw yellow to amber yellow.

*Turnip.* On steamed turnip cylinders growth is similar to that on carrot. The cylinder is entirely covered after three days with wet-shining, slimy-looking growth, which may be flat and smooth or somewhat raised, standing out on the surface in large drops. The colour is cream to straw yellow.

*Parsnip.* On steamed cylinders of parsnip, growth is similar to that on other vegetable cylinders described but rather less copious. The colour is straw yellow to amber yellow.

*Beet.* A very copious, spreading, wet-shining growth is also obtained on steamed cylinders of beet; the growth is more raised than on the other media and straw yellow in colour. On all steamed vegetable cylinders the amount of growth varies with the amount of moisture present, the best growth being obtained with the maximum amount of moisture.

*Steamed rice.* The rice grains become thinly covered with a spreading wet-shining growth which is straw yellow on the wetter parts of the medium and becomes light ochraceous buff where there is less moisture.

*Sweet potato* was a very favourable medium, growth being similar to that on the ordinary potato tubes.

*Streak cultures on Loëffler's blood serum.* Growth was fairly abundant, in the form of a wet-shining, cream-coloured streak along the needle track; liquefaction was observed on the sixth day and proceeded slowly.

*Nutrient bouillon* +15. Growth in bouillon varies considerably with the reaction of the medium. In tubes with a reaction of +15 of Fuller's scale the bouillon is faintly clouded after 48 hours; it never becomes turbid. A straw yellow ring 1–2 mm. broad forms above the liquid and there are often minute, whitish flocculent particles suspended in the liquid which eventually sink and form a sediment. In + 20 bouillon there is a slight pellicle formed, which sinks when disturbed, and considerable sediment is produced.

*Durham's solution* becomes lightly clouded. There is no ring, but often a few very minute flocculi in suspension which eventually sink and form a slight sediment.

*Uschinsky's solution.* There is no growth in this medium; it was kept under observation for 20 days.

*Cohn's solution.* No growth.

*Egg albumen medium* containing 1 gm. of powdered egg albumen and 50 c.c. of a ·05 per cent. solution of potassium phosphate became well clouded, but there was no ring or pellicle formation and no discolouration of the medium.

*Fermi's solution* becomes lightly clouded on the second day. Growth proceeds slowly and after about three weeks there is a good ring above the medium, and considerable sediment at the bottom of the tube. This is Naples yellow in colour and very viscid, rising in a spiral swirl when the tube is rotated.

*Cabbage broth* clouds fairly heavily: a ring forms above the medium and there is some indication of pellicle formation; there is also a sediment at the bottom of the tube.

*Milk* shows no change until the third day; separation of the whey takes place slowly in such a way that there is an increasing quantity of clear yellowish whey at the surface, and the lower part of the tube is filled with whitish minute flocculent particles. The clot is not coagulated, and is slowly digested, but had not completely disappeared in cultures which had been under observation for some weeks. The whey is clear and yellowish in colour.

In litmus milk the colour is partially reduced, and the culture becomes slightly more acid than the control. In flasks, where a greater part of the medium is exposed to the air the action of the bacterium is considerably more rapid than in tubes.

### *Physical and Biochemical Relations.*

*Proteolytic activity in milk.* In describing the cultural characters of the organism, it has been stated that milk is slowly peptonised. A number of flasks each containing 50 c.c. of milk were tested at intervals of five days for peptone, tyrosine and tryptophane. At the end of five and ten days at 30° C. the culture fluid gave positive reactions for each of these compounds. In each case the reaction was stronger on the 15th and 20th day, particularly in the case of tyrosin for which an intense reaction was obtained on the 20th day. There was also a strong reaction for peptone on the 20th day.

A similar set of flasks was tested quantitatively for the production of amino-acids and ammonia. The following figures give the results from one such experiment.

*Ammonia by distillation.*

| | Number of c.c. $N/10$ acid neutralised | | | N as ammonia |
|---|---|---|---|---|
| | Culture | Control | – Control | approx. figure |
| 5 days | 4·27 | 3·40 | ·87 | ·0022 grm. |
| 10 „ | 14·14 | 3·07 | 11·07 | ·0155 „ |
| 15 „ | 24·90 | 2·80 | 22·10 | ·0309 „ |
| 20 „ | 35·33 | 4·76 | 30·57 | ·0428 „ |

From these figures it will be seen that the amount of ammonia increases steadily up to the 20th day. Similar results were obtained from other tests carried out on the same lines.

The amount of amino-acids was estimated by the Sorensen method; it increases up to the 15th day and then decreases, the amino-acids being probably broken down to ammonia.

| | Amount of $N/10$ NaOH used for final titration | | | | N as |
|---|---|---|---|---|---|
| | Culture | Control | – Control | – Ammonia | amino-acids |
| 5 days | 9·57 | 8·33 | 1·24 | ·37 | ·00005 grm. |
| 10 „ | 34·42 | 9·61 | 24·81 | 13·74 | ·0192 „ |
| 15 „ | 47·08 | 4·00 | 43·08 | 20·98 | ·0294 „ |
| 20 „ | 48·42 | 8·27 | 40·14 | 9·58 | ·0134 „ |

In *egg albumen* medium (1 gm. egg albumen in 50 c.c. of 0·5 per cent. potassium phosphate) similar results were obtained. The qualitative test showed that less tyrosin was formed than in the milk cultures, but a strong reaction for peptone was obtained all through.

The culture and control were tested for amino-acids and ammonia in the same way as the milk flasks. To an additional flask, zinc sulphate was added to complete saturation, and the total nitrogen in the filtrate determined by the Kjeldahl process; this gave nitrogen as peptone plus amino-acids and ammonia, and by subtraction nitrogen as peptone. It will not be necessary to give detailed figures as in the previous schedules; taking the amount of nitrogen in 1 gm. egg albumen to be ·15 gm. a typical test gave the following figures. In each case the control was tested and figures obtained from control flasks deducted.

*Percentage of egg albumen reduced.*

| | Total | Ammonia | Amino-acids | Peptone |
|---|---|---|---|---|
| 5 days | 44·5 | 4·4 | 6·0 | 36·0 |
| 10 „ | 51·6 | 8·1 | 13·2 | 30·4 |
| 15 „ | 63·9 | 15·1 | 2·8 | 46·0 |
| 20 „ | 82·2 | 15·0 | 1·2 | 66·0 |

The amount of ammonia and peptone increases gradually; the amino-acid, as in the case of the milk flasks, increases and then decreases, the decrease being correlated with an increase in the ammonia content. The large percentage of albumen reduced shows that the organism is a fairly active proteolytic agent.

A fair amount of ammonia is also produced in media containing peptone, *e.g.* ordinary nutrient bouillon.

Broth cultures after sterilisation cause no peptonisation of milk. If a culture is killed by exposing it to a temperature of about 55° C. for half-an-hour, and then 3–5 c.c. of the culture run into each of a number of tubes of litmus milk, there is no change in the milk during ten days. *Bacterium nectarophilum* gave positive results with this test, the milk being slowly cleared in the same way as when the organism was growing in the medium.

*Amylolytic action.* The starch of steamed potato cylinders is not destroyed at all rapidly; cylinders on which the organisms had been growing for three weeks still gave a strong blue black reaction with iodine.

Tubes containing 10 c.c. nutrient bouillon and ·01 gm. soluble starch were planted with a vigorous culture and incubated at 30° C. It was only after 14 days that the starch entirely disappeared. When a similar set of tubes was planted with *Bacterium citri* the starch had entirely disappeared at the end of 48 hours; the amylolytic action of the tomato organism is therefore comparatively feeble.

Cultures in nutrient broth which had been incubated for five days at 30° C. were tested for the presence of diastase. A mixture was made of equal quantities of the cultivation and a thin starch paste containing 2 per cent. thymol; this was placed in the incubator at 37° C. for six hours and then tested with Fehling's solution, with negative results. Control cultures of *Bacillus subtilis* tested in the same way gave a good reaction for reducing sugars.

*Production of invertase.* Nutrient bouillon in which the organism had been growing for five days was tested for the presence of invertase. A mixture was prepared containing equal quantities of the cultivation and a 2 per cent. solution of cane sugar to which 2 per cent. of carbolic acid had been added. After several hours the mixture was tested for reducing sugars with negative results.

When the organism is grown in nutrient broth containing cane sugar, however, a certain amount of reducing sugar is produced. In flasks containing 500 c.c. of nutrient broth with 2 per cent. saccharose, the

organism produced 61 mg. of reducing sugar in three days, and at the end of five days the culture contained 159 mg. of reducing sugar.

*Gas formation.* No *gas* was produced in fermentation tubes containing nutrient broth and 2 per cent. of following substances: dextrose, laevulose, saccharose, maltose, lactose, mannite, dextrin, galactose, glycerine. In no case was any growth observed in the closed end of the tube. There was a small amount of alcohol in the distillate from cultures in dextrose bouillon. In tube cultivations in iron and lead peptone solution and broth, there was a slight discolouration of the precipitate after three weeks, showing that traces of sulphuretted hydrogen are liberated.

*Acid production.* The behaviour of the organism in the various sugar broths very closely resembles that of *Bacterium campestre* and other organisms of this group, that is to say, it produces a small amount of acid from certain sugars but the acid production is very rapidly obscured by the formation of alkali, probably in the form of ammonia, which has shown to be produced in appreciable quantities in peptone broth.

In dextrose bouillon the acidity is more marked than in any of the other media, the greatest acidity being observed about the fifth day; the acidity of the medium gradually decreases and after three or four weeks it becomes neutral or slightly alkaline.

The same may be said of cultures in nutrient bouillon with 2 per cent. laevulose, saccharose, lactose, galactose, glycerine, and dextrin, but less acid is produced with any of these substances than with dextrose. No definite acid formation was observed in flasks containing mannite and maltose.

*Indol and phenol production.* Cultures in nutrient bouillon and Dunham's solution were repeatedly tested for indol on the third, fifth and tenth day, but with negative results. No reaction was obtained with a nitrite and sulphuric acid, nor with the Rosindol test.

Cultures in nutrient bouillon tested on the tenth day also gave a negative reaction for phenol.

*Reducing agent formation: colour destruction.* A number of tube cultivations were prepared in nutrient bouillon tinted with litmus, rosolic acid, neutral red, methyl orange, and methylene blue: no colour reduction was observed.

*Nitrate reduction.* A number of tube cultivations in nitrate bouillon were tested after three, five and ten days. Some of these showed a trace of nitrite but in others no reaction was obtained. The controls gave no reaction for nitrite.

Flasks of nitrate bouillon tested after ten days contained slightly more ammonia than similar flasks containing bouillon without potassium nitrate. These tests were repeated several times with no more decided results; it would seem therefore that the organism has a feeble action on nitrates, and that traces of nitrite and ammonia are at times produced in culture solutions containing a nitrate.

*Atmosphere.* The organism is strictly aerobic and makes no growth in the depth of media nor in the absence of oxygen. No growth took place in Bulloch's apparatus in an atmosphere of hydrogen, nitrogen or carbon dioxide, but except in the case of tubes exposed to $CO_2$ the organism was not killed and began to grow when removed from the apparatus and placed in the incubator.

*Temperature.* This bacterium grows through a wide range of temperature, it grows slowly at 5° C. and at 40° C.; the optimum is about 30° C. It is killed by a prolonged exposure to 42° C. The thermal death point (moist, ten minutes' exposure) is 56° C.; in dry tubes the organism did not grow after ten minutes' exposure to a temperature of 64° C.

*Reaction of medium.* This bacterium does not grow at all in an alkaline bouillon, even if only − 5 of Fuller's scale; it grows very slowly in bouillon neutral to phenol-phthalein. It grows well in bouillon with a natural reaction of + 25, the optimum being about + 20 of Fuller's scale; it grows almost as well at + 15 as at + 20. A number of cultures were also made in broth which had been made neutral and acidified with various acids. It grew well in + 20 (Fuller), malic, lactic, tartaric, hydrochloric and citric acids; in tubes with a reaction of + 25 Fuller, it grew in tubes acidified with malic, lactic and citric acids and not with tartaric or hydrochloric acids; it is much less sensitive to malic and citric acids, growing unrestrainedly in bouillon with a reaction of + 30 Fuller, and clouding bouillon up to + 50 Fuller, malic acid and + 60 citric acid; the inhibition point for these was + 55 and + 65 respectively.

*Toleration of sodium chloride.* Growth is inhibited by 4 per cent. of sodium chloride; the organism grows freely in nutrient bouillon containing 3 per cent. sodium chloride.

*Resistance to fungicides.* Tests were made in nutrient bouillon containing various percentages of copper sulphate, phenol, mercuric chloride and formalin. Tubes were planted fairly heavily and after ten minutes a plate was poured from each dilution. In this way the inhibition coefficient and lethal coefficient were determined.

The organism is killed by ten minutes' exposure to copper sulphate

1 : 25; phenol 1 : 500; mercuric chloride 1 : 500 and formalin 1 : 200. It is extraordinarily resistant to copper sulphate, the lethal coefficient for *Bacillus mangiferae* being about 1 : 400. The inhibition coefficient was not determined very exactly; the bacterium grows in a bouillon containing 1 : 1000 copper sulphate, but was inhibited by 1 : 800; the same result was obtained with formalin. The organism grows in bouillon containing phenol 1 : 800 but not in 1 : 600; similarly it grows in mercuric chloride 1 : 3000 but not in 1 : 2500.

*Desiccation.* A number of sterile cover slips were covered with a film of the organism and placed in sterile ventilated petri-dishes in a desiccator; one of these was removed each day and dropped into a tube of nutrient bouillon. No growth was obtained from cover slips which had been exposed to desiccation for more than ten days.

## Nomenclature.

The name *Bacterium vesicatorium* n. sp. is suggested for this organism, which has apparently not previously been described: its chief characters are as follows:

*Bacterium vesicatorium* n. sp. A parasite of the tomato plant, causing spots on leaves and stems and raised blisters or cankers in the fruit. A motile rod averaging 1–1·5 × ·6–·7 $\mu$, with rounded ends and a single polar flagellum, occurring singly, in pairs, or in short chains. On nutrient agar colonies appear in 48 hours at 30° C. and are finally circular, about 5 mm. diameter, semi-translucent, Naples yellow in colour. On potato produces a copious yellow, spreading growth, butyrous or rather viscid in consistency. Liquefies gelatine and blood serum; no growth in Uschinsky's and Cohn's solutions: grows slowly but well in Fermi's solution. In milk there is a separation of whey, and casein becomes slowly peptonised. Is a fairly active proteolytic agent, destroys starch very slowly. There is no gas formation in sugar bouillon; there is a slight increase in acidity but bouillon ultimately becomes more alkaline. No definite evidence of nitrate reduction was obtained; no indol or phenol is produced in bouillon or Dunham's solution. The organism is strictly aerobic; grows through a wide range of temperature from 5° C. to 40° C.; the optimum temperature for growth is about 30° C.; thermal death point 56° C. Does not grow in alkaline media, but tolerates a fair amount of acid; growth inhibited by 4 per cent. sodium chloride. Group number 211, 2332523.

Two other yellow organisms have been described as causing diseases

of the tomato, *Aplanobacter michiganense* Erw. Sm.(2) and an organism believed to be identical with *Bacillus Lathyri* Manns and Taubenhaus(1).

*Aplanobacter michiganense* is non-motile, tolerates considerable alkali, growing in − 25 and − 30 peptone bouillon and does not grow in peptone beef bouillon acidified to + 20, + 25, or + 30 with malic or citric acid.

The second organism has four to six peritrichous flagella and grows in Uschinsky's solution: cultures in nitrate broth give a strong reaction for nitrite on the second day.

The three organisms appear to be quite distinct, and *Bacterium vesicatorium* differs considerably from the other two in its effect on the host.

## CONTROL OF THE DISEASE.

The organism is not very sensitive to the fungicides usually employed in spraying solutions, and experience has shown that spraying is of little use in combating plant diseases caused by bacteria. This is particularly the case with organisms disseminated chiefly by rain splash and infecting the plant through the stomata.

It appears to be the custom of the market gardeners in the Pretoria district to save seed from their own plants for the following season. This custom is probably in part responsible for carrying over the disease from one season to another. Certain varieties are more susceptible than others, but it has been found practically impossible to discover the name of the varieties usually grown. The following methods are therefore suggested for the control of the disease.

(1) Selection of resistant varieties.

(2) Sterilisation of the seed by means of formalin or mercuric chloride.

(3) A long crop rotation.

(4) Destruction of diseased fruit and of affected plants at the end of the season.

In connection with (3) it will also be of importance that the irrigation furrows shall not be allowed to flow through the old tomato bed to the site selected for tomatoes in the following season.

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## EXPLANATION OF PLATE XXVI

Fig. 1. Tomato fruits severely attacked by *Bacterium vesicatorium.*
Fig. 2. A milder form of the canker showing the characteristic blistering in early stages of infection.

Fig. 1.

Fig. 2.

# PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

*September 24th*, 1920.

PAPERS.

I. The Relation of Climatic Factors to Disease in Plants. E. S. Salmon.

II. Immunity to Disease in Plants as a Mendelian Factor. F. T. Brooks.

III. The Relation of Soil Aeration and Soil Temperature to Disease in Plants. A. Howard.

*November 5th*, 1920.

PAPERS.

I. The Reclamation of Waste Land by Botanical Means. F. W. Oliver.

II. The Reclamation of Waste Land by Agricultural Means. E. J. Russell.

*December 10th*, 1920.

EXHIBITS.

I. Cultures of Soil-Algae from the Rothamsted Plots. B. Muriel Bristol.

II. (*a*) A New Method of Flagella Staining.
(*b*) A New Incandescant Lamp for Microscopic Use. S. G. Paine.

III. *Phomopsis pithya* Lind., on Douglas Fir. N. L. Alcock.

IV. Photograph of E. F. Smith of Washington. Wm B. Brierley.

PAPERS.

I. Problems of Economic Biology in British East Africa. W. J. Dowson.

II. Nitrogen Fixation in the Ericaceae. M. C. Rayner.

*January 28th*, 1921.

EXHIBIT.

I. *Achatina fulica*. E. E. Green.

PAPERS.

I. Greenhouse Whitefly and its Control. R. L. Lloyd.

II. Personal Impressions of some American Biologists and their Problems. Wm B. Brierley.

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